MORPHOLOGICAL SPECTRUM

OF

LUPUS NEPHRITIS

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Submitted By

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Dedicated

to

my Family,

friends

&

Teachers

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Dr. RAMNEEK KAUR

ABBREVIATIONS

ANCA	Anti Neutrophil Cytoplasmic Antibody
ANA	Antinuclear Antibodies
DPX	Dibutylphthalate Polystyrene Xylene
DIF	Direct Immunofluorescence
ELISA	Enzyme Linked Immunosorbent Assay
ESRD	End Stage Renal Disease
EULAR	European League Against Rheumatism
H&E	Hematoxylin & Eosin
ISN/RPS	International Society of Nephrology/Renal Pathology Society
IFTA	Interstitial Fibrosis and Tubular Atrophy
LN	Lupus Nephritis
LE	Lupus Erythematosus
MT	Masson Trichrome
MPO	Myeloperoxidase
PAS	Periodic Acid Schiff
PBS	Phosphate buffer solution
SLE	Systemic Lupus Erythematosus

ABSTRACT

Introduction: Renal disease manifests in up to 40-75% of SLE patients, most often occuring within five years of disease onset and hence is considered one of the strongest predictors of an unfavourable outcome. Renal biopsy at the time of initial presentation and subsequently during follow up may be needed to decide the therapeutic approach. Histopathological features play a significant role in patient management.

Aims & Objectives:

1. To study the histopathological features of kidney biopsies in patients of SLE.
2. To correlate the histopathological features with the clinical profile

Materials and Method: This study was conducted in the Department of Pathology at Dayanand Medical College and Hospital, Ludhiana for a period of 2 years, of which 1 year (1st March 2020-28th Feb 2021) was retrospective and 1 year (1st March 2021-28th Feb 2022) was prospective. All the biopsies were histologically analysed and classified into various classes based on light microscopy findings using routine, special stains and direct immunofluorescence. Correlation with indirect immunofluorescence findings was done whenever possible. In the retrospective analysis, the slides were retrieved from the archives along with their clinical history and immunofluorescence finding.

Results: The cases analysed had a mean age of 38.2±14.5 years. Highest number of cases belonged to the age of 21-40 years. Male to Female ratio of 1:4.4 were observed. The most common clinical presentations included synovitis, oedema and fever. Majority of the cases belonged to class IV LN. Among the hematological parameters anaemia was most common. Full house positivity was observed in 66.6% cases.

Conclusion: The renal biopsy represents the gold standard in management of LN. Studies performed so far show an overall greater reproducibility of the ISN/RPS classification than previous classifications.

Keywords: Lupus Nephritis, Kidney Biopsy, EULAR classification, ANA, Direct immunofluorescence.

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INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune condition that affects multiple organ systems and can present with alternating periods of relapse and remission. The word "lupus" comes from the Latin word for wolf, and refers to the ulcerative skin lesions in patients that resemble a wolf bite. SLE was first described in detail by William Osler in 1899, who also identified nephritis as one of its components. The underlying cause of SLE is not well understood, but it is thought to be a combination of genetic and environmental factors. Symptoms can vary widely, including joint pain, skin rashes, fatigue, fever and renal problems. [1]

The kidneys are frequently involved in SLE, and the decline in renal function is due to various lesions in the glomerulus, tubules, and vasculature. Lupus Nephritis (LN) is present in approximately 40% of SLE patients, usually within five years of diagnosis. Sadly, LN still carries a progression rate of 4.3-10.1% per year towards End Stage Renal Disease (ESRD) [2]

Approximately 50% of patients with SLE develop Lupus Nephritis, which is the leading cause of kidney damage in SLE. However, it is not the only cause. Males with SLE tend to have a more severe form of the disease, including higher rates of kidney and cardiovascular involvement, and a higher likelihood of developing kidney failure, compared to females with SLE. [3]

Lupus Nephritis can present different clinical symptoms, as well as varying ratios of normal nephrons, acute inflammatory lesions, and chronic damage at different times in the same patient. This heterogeneity of LN is not only observed between patients, but also within the same patient at different stages of the disease. While rapid nephron damage can occur during acute inflammation in LN, it can be reversed with proper immunosuppressive treatment. On the other hand, chronic damage such as fibrosis and tubular atrophy does not respond to immunosuppressive treatment and leads to long-term renal impairment. [4]

Antinuclear antibodies (ANA) are the hallmark of the disease and are usually the initial test performed. Immunofluorescence assay is considered the gold standard test for ANA, and although other methods of detection such as ELISA and multiplex assays are widely available, they lack sensitivity. Anti-Ds-DNA antibodies have more than 95% specificity for SLE but are seen in only about 60% to 70% of SLE patients. Anti-Ro (SSA) and Anti-La (SSB) antibodies target ribonucleoprotein particles. Anti-Ro and Anti-La antibodies are seen in up to 90% of cases of Sjogren syndrome but can be seen in SLE as well. Anti-Smith antibodies are seen in less than 30% of SLE patients but have 99% specificity for SLE.[5]

According to the European League Against Rheumatism and European Renal Association–European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations, in patients with GFR below 30 ml/min, the decision for biopsy becomes doubtful unless the kidney size is normal (> 9 cm length in adults) and/or there is evidence of renal disease activity. [6]

Due to the varied histopathology of Lupus Nephritis, multiple classifications have been proposed in the past four decades. The earliest one was introduced by the World Health Organization (WHO) in 1974 and was later refined by Austin and colleagues. To enhance standardization, eliminate inconsistencies and ambiguities, and promote uniform reporting, the International Society of Nephrology/Renal Pathology Society (ISN/RPS) introduced a new classification in 2003. This classification more precisely describes all classes of Glomerulonephritis (GN) and clearly defines criteria for activity and chronicity. [7]

AIMS AND OBJECTIVES

1. To study in detail the histopathological features of kidney biopsies in patients of SLE.
2. To correlate the histopathological features with the clinical profile.

REVIEW OF LITERATURE

The kidneys are paired retroperitoneal organs that extend from the 12th thoracic vertebra to the 3rd lumbar vertebra with the right kidney lying at slightly lower level than the left kidney. The normal weight of the kidneys range from 125 gm to 170 gm in adult males and 115 gm to 155 gm in adult females.[8]

The kidney performs three major functions for the body. The most important being the excretory function by converting more than 1700L of blood per day into 1 L of highly concentrated urine and therefore excreting the waste products of metabolism. Along with maintaining the body's concentration of water, salt, calcium, phosphorus and other cations and anions, the kidneys also secrete hormones such as erythropoietin, renin and prostaglandins and thus performing the endocrine functions.[9]

Histologically, the kidneys are composed of three major components: the cortex, the medulla and the collecting system. The renal parenchyma includes the nephron, blood vessels, lymphatics and the interstitium. Nephron forms the functional and structural unit of the kidney which includes a renal corpuscle (composed of Bowman's capsule and glomerulus) and renal tubule. Arising from the cortex, it loops down into the medulla and then returns to the cortex. From here they drain into the collecting ducts and descend into the medulla from the apex of the medullary pyramids. The funnel shaped renal pelvis envelopes the apical part of the pyramid, known as the renal papilla. Each renal papilla is surrounded by a branch of the renal pelvis called a calyx. The renal medulla is composed of multiple medullary pyramids. The renal tubules include the proximal convoluted tubules, loop of Henle, the distal convoluted tubule and the collecting ducts.[10]

The function of filtering the plasma is performed by the renal corpuscle which is composed of Bowman's capsule and the glomerulus. The Bowman's Capsule is the dilated proximal most extension of the proximal tubule. It is lined by parietal epithelial cells on its inner aspect within which the glomerular tuft is located.

The glomerular tuft floats within a fluid filled cavity known as the Bowman's space. The glomerulus is a tuft of tiny blood vessels in the kidney that acts as a filter for waste products, and its average diameter in the adult human is approximately 200 μm.[8] The glomerulus is formed by a global network of anastomosing capillaries which project into Bowman's capsule. The glomerular capillary loops are lined by podocytes which form the visceral layer of epithelium. Podocytes, the largest cell of the glomerulus, participate in glomerular basement membrane synthesis and are the principal cells in the permselectivity qualities of the capillary loop. The visceral layer becomes continuous with the parietal layer that constitutes Bowman's capsule proper.

The glomerular capillary wall is the filtering membrane known as the filtration barrier. The filtration barrier is formed by a thin layer of fenestrated endothelial cells, the glomerular basement membrane and visceral epithelial cells also known as the podocytes. The glomerular basement membrane is 240-340 nm wide.[8] Electron microscopy reveals the basement membrane as having three layers: a central, thick, and electron-dense layer called the lamina densa, and two less-dense layers beneath the epithelial and endothelial cells, known as the lamina rara externa and lamina rara interna, respectively.

The mesangium supports the tufts of glomerular capillaries, which are the intraglomerular extensions of the arteriolar walls. The two components of the mesangium include; the extracellular mesangial matrix and the cells of the mesangium known as the mesangial cells. The mesangial matrix also has a large number of extracellular matrix proteins including the various types of collagens (III, IV, V, VI), fibronectin, proteoglycans. The mesangial cells perform numerous functions which include contraction, production of extracellular matrix along with secretion of mediators of inflammation.

The inner surface of the glomerular capillary loops are lined by the endothelial cells. Endothelial cells have fenestrated regions and non-fenestrated regions. The proteoglycans of the glomerular basement membrane are negatively charged creating a charge selective barrier

to the glomerular filtrates. The basement membrane along with the fenestrated endothelial cells also form a size selective barrier.

The renal tubules are divided into four distinct histophysiological zones: proximal tubules, loop of Henle, the distal convoluted tubule and the collecting ducts while extending from the Bowman's capsule to its junction with the collecting ducts.

Interstitium forms the support tissue of renal parenchyma which corresponds to the space between the glomeruli, tubules, vessels and nerves. It is composed of cells consisting of fibroblasts along with the cells of the immune system e.g monocytes, macrophages and lymphocytes and the matrix which is formed by proteoglycans, glycoproteins and fluids.

The main renal artery arises from the aorta and divides into segmental arteries. The segmental arteries divide within the renal sinus to form the interlobar arteries which give way to arcuate arteries located between the cortex and the medulla. Each set of arcuate arteries run between the septa of Bertin and the medullary tissue in the pyramids. These arcuate arteries give rise to afferent arterioles. The peritubular capillary plexus is formed by efferent arterioles, which originate from the glomerulus. Blood is supplied to the medulla via these efferent arterioles.

SLE is a chronic autoimmune disease in which the body's immune system attacks its own tissues and organs. The cause of this condition is unknown and it affects multiple organ systems.[11] Majority of the patients are women of child bearing age; while people of all genders, ages and ethnic groups are susceptible.[12] The presence of autoantibodies to nuclear constituents, known as antinuclear antibodies, is a defining feature of the disease. These antibodies and immune complexes play a role in causing damage to the organs. In many cases, the autoantibodies can be detected several years before the appearance of clinical symptoms.[12] The manifestation and progression of SLE vary greatly from patient to patient. Some may experience periods of spontaneous improvement, while others may have mild musculoskeletal symptoms that respond to treatment. In

severe cases, the disease can progress rapidly and affect multiple systems, leading to death despite immunosuppressive therapy.[12] The most common organs affected by SLE include skin, joints, kidneys, serosal surfaces including pleura and pericardium, CNS and hematopoietic system. Lupus nephritis, characterized by inflammation and damage to the kidneys, is a frequent manifestation of SLE.

SLE patients produce a variety of autoantibodies. Antinuclear antibodies are present in over 90% of patients and are the most commonly used test for SLE, although they are not specific to the disease. Anti-dsDNA antibodies are more specific markers for SLE, but they are less frequently detected in patients compared to ANA.[13]The presence of high levels of anti-dsDNA antibodies is indicative of increased disease activity in SLE patients, particularly in those with lupus nephritis.[13] In cases of active SLE and active lupus nephritis, the serum levels of complement components C3 and C4 are typically low. Many patients with active proliferative lupus nephritis have high levels of anti-dsDNA antibodies and low levels of C3 and C4.[14]

Table 1- Systemic lupus international collaborating clinic criteria for SLE (2018) [12]

<table>
<tr><th>CLINICAL MANIFESTATIONS</th><th>IMMUNOLOGIC MANIFESTATIONS</th></tr>
<tr><td>SKIN-
● Acute, subacute cutaneous LE (photosensitivity, malar, maculopapular, bullous)
● Chronic cutaneous LE (discoid lupus, panniculitis, lichen planus-like, hypertrophic verrucous, chillblains)</td><td rowspan="3">● ANA > reference negative value
● Anti- dsDNA > reference, if by ELISA 2x reference
● Anti-Sm antibodies
● Antiphospholipid antibodies (any of lupus anticoagulant, false positive RPR, anti cardiolipin, anti beta glycoprotein I)</td></tr>
<tr><td>Oral or nasal ulcers</td></tr>
<tr><td>Nonscarring Alopecia</td></tr>
</table>

Synovitis involving ≥ 2 joints	● Low serum complement (C3, C4 or CH50) ● Positive direct Coombs test in absence of hemolytic anemia
Serositis (pleurisy, pericarditis)	
Renal- Urine Prot/Cr ≥ 0.5 RBC casts	
Neurologic- Seizures, psychosis, mononeuritis, myelitis, peripheral or cranial neuropathies, acute confusional state	
Hemolytic anemia	
Leucopenia (<4000/μL) or Lymphopenia (<1000/μL)	
Thrombocytopenia (<100000/μL)	

Presence of any four criteria (must have at least 1 in each category) qualifies patient to be classified as having SLE [12]

EPIDEMIOLOGY OF SLE:

Systemic lupus erythematosus (SLE) affects 1 in every 2,500 cases, with a higher incidence among women of childbearing age (17-55 years) where the female to male ratio is 9:1. However, this gender predominance is not as pronounced in children and older individuals. The prevalence of SLE is 2 to 3 times higher in Black and Hispanic populations compared to whites. Regarding renal disease, both males and females have equal incidences. Lupus nephritis is more severe in children and males and less likely in the elderly population.[9] It is a common and serious complication of SLE and most severe in African-American adolescents. [15]

ETIOLOGY AND PATHOGENESIS OF SLE:

The underlying cause of SLE is a result of the interaction between genetic, environmental and hormonal factors. Multiple genes have

been linked to SLE, which is indicated by the clustering of cases in families, the high concordance rate of SLE in identical twins (over 25%), and the frequent presence of positive autoantibodies and autoimmune disorders in family members of SLE patients. Certain human leukocyte antigen (HLA) genotypes, such as HLA B8, DR2, and DR3, carry a higher risk for the development of lupus.

The environmental factors that have been identified as contributing to the onset of SLE include exposure to UV light, Epstein-Barr virus, prolonged occupational exposure to silica dust, and current smoking.[12] Additionally, UV light exposure has been observed to trigger and worsen SLE and lupus nephritis, potentially due to increased apoptosis (cell death) in keratinocytes.[16,17] EBV antigens by antigen mimicry may also trigger SLE in susceptible individuals. The strong female predominance of SLE and its exacerbation in response to oral contraceptive pills containing estrogen and hormone replacement therapy suggest a role for hormonal factors in the development and progression of the disease.[17] Binding of estradiol to receptors on B and T lymphocytes stimulates activation and extends the survival of these cells, resulting in prolonged immune responses and elevated antibody production.

The loss of self-tolerance and development of autoimmune responses in SLE is caused by abnormalities in immune regulation. Defective autoregulation of T cells, exposure to self-antigens, hyperactivity of self-antigen driven T cells, and polyclonal proliferation of B cells caused by increased B cell stimulating cytokines may contribute to the production of autoantibodies.[17] These autoantibodies are directed against nucleic acids, nucleosomes (DNA-histone complex), chromatin antigens and small nuclear and cytoplasmic ribonuclear proteins.

MECHANISMS OF TISSUE INJURY:

The majority of the systemic manifestations of SLE are due to tissue injury caused by immune complexes, which is a type III hypersensitivity reaction. The low levels of serum complement observed in SLE patients is a result of the consumption of complement proteins and the

accumulation of complement and immunoglobulin deposits in the glomeruli. T cell infiltrates are also commonly observed in affected tissues. Nuclei of damaged cells in these tissues react with antinuclear antibodies (ANAs), leading to loss of their chromatin pattern and becoming homogenous, resulting in the formation of LE bodies, also known as hematoxylin bodies.[9]

Cytopenias result from the opsonization caused by autoantibodies leading to phagocytosis and destruction of the red blood cells, white blood cells and platelets. These are examples of antibody-mediated (type II) hypersensitivity. Individuals with antiphospholipid antibodies are at risk of developing both venous and arterial thrombosis, which can be associated with recurrent spontaneous miscarriages and focal cerebral or ocular ischemia. Neuropsychiatric manifestations result from the antibodies crossing the blood brain barrier react with neurons or receptors of various neurotransmitters.

Table 2-Autoantibodies in SLE [12]

ANTIBODY	PREVALENCE (%)	ANTIGEN RECOGNISED	CLINICAL UTILITY
Antinuclear antibodies	98	Multiple nuclear antigens	Best screening test; repeated negative tests make SLE unlikely
Anti-dsDNA antibodies	70	DNA (double - stranded)	High titers are SLE- specific and in some patients correlate with disease activity, nephritis, vasculitis
Anti-Sm antibodies	25	Protein complexed to 6 species of nuclear U1 RNA	Specific for SLE; no definite clinical correlations; most patients also have anti- RNP; more common in blacks and Asians than whites

Anti-RNP antibodies	40	Protein complexed to U1 RNA	Not specific for SLE; high titers associated with syndromes that have overlap features of several rheumatic syndromes including SLE; more common in blacks than whites
Anti-Ro (SS-A) antibodies	30	Protein complexed to hY RNA, primarily 60 kDa and 52 kDa	Not specific for SLE; associated with sicca syndrome, predisposes to subacute cutaneous lupus, and to neonatal lupus with congenital heart block; associated with decreased risk for nephritis
Anti-La (SS-B) antibodies	10	47-kDa protein complexed to hY RNA	Usually associated with anti-Ro; associated with decreased risk for nephritis
Antihistone antibodies	70	Histones associated with DNA (in nucleosome, chromatin)	More frequent in drug- induced lupus than in SLE

Antiphospholipid antibodies	50	Phospholipids, β2glycoprotein 1 cofactor, prothrombin	Three tests available—ELISAs for cardiolipin and β2 glycoprotein 1, sensitive prothrombin time (DRVVT); predisposes to clotting, fetal loss, thrombocytopenia
Antierythrocyte antibodies	60	Erythrocyte membrane	Measured as direct Coombs' test; a small proportion develops overt hemolysis
Antiplatelet antibodies	30	Surface and altered cytoplasmic antigens on platelets	Associated with thrombocytopenia but sensitivity and specificity are not good; this is not a useful clinical test
Antineuronal (includes anti-glutamate receptor) antibodies	60	Neuronal and lymphocyte surface antigens	In some series a positive test in CSF correlates with active CNS lupus.
Antiribosomal P antibodies	20	Protein in ribosomes	In some series a positive test in serum correlates with depression or psychosis due to CNS lupus

MORPHOLOGICAL CHANGES IN SLE[9]

BLOOD VESSELS: An acute necrotizing vasculitis that affects capillaries, small arteries, and arterioles may also occur in SLE. This type of vasculitis is characterized by fibrinoid necrosis of the vessel

walls and, in later stages, fibrous thickening and narrowing of the lumen.

SKIN: SLE is known for its characteristic butterfly rash on the face that affects the nose and cheeks, as well as other skin lesions such as urticaria, bullae, maculopapular lesions, and ulcerations. Histological examination reveals vacuolar degeneration of the basal layer of the epidermis and variable edema and perivascular inflammation in the dermis. Vasculitis and fibrinoid necrosis may also be present. Immunofluorescence studies show deposition of immunoglobulins and complement along the dermoepidermal junction.

CNS: Noninflammatory occlusion of small vessels by intimal proliferation is sometimes seen. However no clear morphological alterations have been noted.

PERICARDITIS: There may be acute, subacute or chronic inflammation of the serosal lining membranes.

CARDIOVASCULAR SYSTEM: Myocarditis and valvular abnormalities may occur. Valvular endocarditis (called Libman Sacks endocarditis) is common. Infiltration by mononuclear cells is less common.

SPLEEN: The common features include splenomegaly, capsular thickening and follicular hyperplasia. The central penicilliary arteries may show concentric intimal and smooth muscle cell hyperplasia producing so called onion-skin lesions.

LUNGS: Pulmonary involvement is common in SLE patients, occurring in 50-70% of cases, and in some cases, it can even be the first symptom to appear in 4-5% of patients. The complications are numerous and can include pleural involvement, ILD, vasculitis, blood clots in the lungs, high blood pressure in the pulmonary arteries, problems with large airways, shrinking lung syndrome, and infections.[18]

KIDNEY: Renal involvement is a significant aspect in SLE, affecting up to 50% of patients. It primarily manifests as glomerulonephritis and tubulointerstitial nephritis. These lesions are a result of immune complexes depositing on the glomerular basement membrane and mesangium.

LUPUS NEPHRITIS

The World Health Organization (1995) categorizes Lupus Nephritis into 6 classes, all with distinct pathological features and significant differences in clinical outcomes. This has a significant role in patient management in adapting a different treatment approach for each class.

- Class I: Minimal mesangial lupus nephritis- immune complex deposition in the mesangium which can be identified by direct immunofluorescence and by electron microscopy. No structural changes by light microscopy.
- Class II: Mesangial proliferative lupus nephritis-Mesangial cell proliferation without involvement of glomerular capillaries. Normal by light microscopy but has mesangial-positive deposits by direct IF changes and positive EM changes within the mesangium
- Class III: Focal lupus nephritis-involvement of fewer than 50% of glomeruli. Focal and segmental proliferative changes by light microscopy, positive direct IF and EM changes
- Class IV: Diffuse segmental or diffuse global lupus nephritis-involvement of >50% of glomeruli. “Wire loop” structures identified on light microscopy are due to subendothelial deposition of immune complexes. Diffuse proliferative glomerulonephritis with positive direct IF and EM
- Class V: Membranous lupus nephritis-diffuse thickening of the capillary walls due to deposition of immune complexes. Features of diffuse membranous glomerulonephritis- subepithelial and mesangial immune deposits (“spike and dome” pattern with silver stain); nephrotic syndrome or severe proteinuria.
- Class VI: Advanced sclerosing lupus nephritis- sclerosis of >90% of the glomeruli and represents end stage renal disease.

ISN/RPS CLASSIFICATION OF LUPUS NEPHRITIS (2003)[19]

Class I: Minimal Mesangial Lupus Nephritis:

- Glomeruli normal by light microscopy (LM), mesangial deposits by immunofluorescence (IF) / electron microscopy (EM)

Class II: Mesangial Proliferative Lupus Nephritis:

● Mesangial hypercellularity and/or mesangial expansion by LM, immune deposits by IF / EM

Class III: Focal Lupus Nephritis:

● Active or inactive focal, segmental or global endo or extracapillary glomerulonephritis involving < 50% of glomeruli, immune deposits are subendothelial

● Subclassified as A (active lesions), A/C (active and chronic lesions), C (chronic lesions)

Class IV: Diffuse Lupus Nephritis:

● > 50% glomeruli with active / inactive diffuse, segmental, or global endo and/or extracapillary glomerulonephritis

● Diffuse subendothelial deposits can cause wire-loop appearance

● A (active lesions), A/C (active and chronic lesions), C (chronic lesions)

● Diffuse segmental if < 50% glomeruli show segmental lesions, diffuse global if > 50% show global lesions

Class V: Membranous Lupus Nephritis:

● Global or segmental involvement, subepithelial immune deposits

● May occur in combination with class III or IV

Class VI: Advanced Sclerosing Lupus Nephritis:

● > 90% globally sclerosed glomeruli

CLASS I: MINIMAL MESANGIAL LUPUS NEPHRITIS

The patients present with mild symptoms of hematuria or proteinuria, or both. With advancements in supportive and more targeted use of immunosuppressive therapy, the prognosis for these conditions is generally favorable. Upon examination of kidney biopsies, light microscopy shows normal glomeruli but examination through direct immunofluorescence or electron microscopy reveals immune deposits in the mesangium.[20]

CLASS II: MESANGIAL LUPUS NEPHRITIS

These patients also present with mild hematuria or proteinuria or both with better prognosis. Histologically, the kidney biopsy contains mesangial proliferative lesions which are seen as mesangial hypercellularity of any degree or mesangial matrix expansion. Direct immunofluorescence shows mesangial deposits.[20]

In both Class I and Class II Lupus Nephritis, immunofluorescence or electron microscopy may show few isolated subepithelial or subendothelial deposits but histologically these kidney biopsies will not have endocapillary/extracapillary proliferation, sclerotic or necrotising lesions. The presence of the latter changes lead to the diagnosis of Class III (if less than half of the glomeruli involved) or Class IV (if more than half of the glomeruli are involved) Lupus Nephritis.[20]

CLASS III: FOCAL LUPUS NEPHRITIS

These patients present with nephritic or nephrotic range proteinuria. This class of lupus Nephritis involves focal proliferative, sclerotic or necrotising lesions. Focal involvement refers to involvement of less than half of the glomeruli available for microscopic examination. The proliferative lesions may be seen as mesangial proliferation and endocapillary proliferation along with presence of inflammatory cells. The necrotizing lesions may be seen as fibrinoid necrosis and crescent formation. Fibrinoid necrosis is seen as an amorphous eosinophilic material which may be highlighted as bright red in trichrome stain. These changes are often seen in active lesions. Silver stain may show double contouring of the glomerular basement membrane. Marked degree of the interstitial inflammatory infiltrate may lead to disruption of the Bowman's capsule, hence rupturing it. Interstitial fibrosis and tubular atrophy may be seen.

On direct immunofluorescence, granular positivity for Immunoglobulin G (IgG), IgM, IgA, C3 and C1q may be seen along the glomerular capillary walls and in the mesangium. Direct immunofluorescence positivity for IgG, IgM, IgA, C3 and C1q is termed as "Full House

Positivity". These are mainly subendothelial deposits which may be confirmed on electron microscopy. Subepithelial deposits may also be seen but are scattered. If there are extensive subepithelial deposits i.e. (>50% glomeruli) the possibility of Class V (Membranous) Lupus Nephritis may be considered.[20]

CLASS IV: DIFFUSE LUPUS NEPHRITIS

These patients have significant proteinuria with active urine sediment and increased renal dysfunction. This is the most common class and a severe form of lupus nephritis. It is characterized by proliferative, necrotising or sclerotic lesions involving more than 50% of glomeruli seen as variable endocapillary hypercellularity and mesangial expansion. Active cases show cellular crescents and necrosis while chronic lesions are seen as segmental sclerosis and fibrocellular to fibrous crescents. Wire-loop lesions are characteristic of Class IV Lupus Nephritis which are seen as PAS positive thickenings of the glomerular capillary walls. These are formed by large subendothelial electron dense deposits. Glomerular lesions may be seen as mesangial hypercellularity, endocapillary hypercellularity or may be even severe forms such as crescents. Tubular atrophy and interstitial fibrosis seen. Arteries may show varying lesions.

On direct immunofluorescence, irregular "Full house positivity" may be seen due to deposits of immunoglobulins and complements along the glomerular capillary walls as well as in the mesangium. These may be highlighted on electron microscopy.[20]

CLASS V: MEMBRANOUS LUPUS NEPHRITIS

Clinically, these patients present with marked proteinuria. It is characterized by diffuse subepithelial deposits involving ≥50% of the loops in ≥50% of the glomeruli. These patients have relatively favorable prognosis.

Histologically, there is variable mesangial expansion with diffuse thickening of the glomerular capillary walls. These thickened capillary walls are highlighted on the Hematoxylin & Eosin (H&E) stain, PAS and

silver stains. On Silver-Methenamine stain, argyrophilic spike formations may be seen along the glomerular capillary loops. These correspond to the subepithelial deposits.

On direct immunofluorescence, granular deposits of immunoglobulins and complements are present along the glomerular capillary walls and in the mesangium. Full house staining of deposits by immunofluorescence helps to distinguish Class V Membranous Lupus Nephritis from idiopathic membranous nephropathy. Also, absence of phospholipase A2 receptor in the deposits proves to be a useful tool in distinguishing idiopathic membranous nephropathy from Class V Membranous Lupus Nephritis.

Combination of Class V with Class III or Class IV Lupus Nephritis has been observed.[20]

CLASS VI: ADVANCED SCLEROSING LUPUS NEPHRITIS

This class is morphologically characterized by >90% glomeruli having global or extensive segmental or global sclerosis without residual activity. Histologically, the features may be similar to other late stages of glomerulonephritis and to focal global sclerotic lesions that occur in the elderly.[20]

VASCULAR LESIONS IN SLE

Various types of vascular lesions are seen in SLE patients. Uncomplicated vascular immune deposits are highly specific for lupus nephritis and are commonly seen. These may be detected by immunofluorescence and electron microscopy within the media of the arteries or arterioles or along the intimal basement membrane. The deposits may consist of immunoglobulins as well as complements i.e. C3c and C1q. However, these are not associated with any particular clinical manifestation.

In patients with severe Lupus Nephritis, a lesion known as Lupus Vasculopathy may occur which is seen as necrosis of arterioles and occasionally large arteries without inflammation. This fibrinoid necrosis is seen as an eosinophilic material with a smudgy appearance within

the intima which may expand the lumen. Lupus vasculopathy has a poorer prognosis.[20] Both hyaline type material with insudated plasma proteins and immune deposits along with fibrin can be documented by electron microscopy.

Vascular smooth muscle cells and endothelial cells may show necrosis, but they are not associated with inflammatory cells i.e. there is not true evidence of vasculitis. Vasculitis is defined as fibrinoid necrosis associated with inflammatory infiltrate through the vascular wall, with or without immune complex deposits.

Thrombotic microangiopathy with or without detectable circulating lupus anticoagulant or antiphospholipid antibodies may also be seen in the patients of SLE. There are no immune deposits in these lesions but they show fibrin within the glomeruli and small arteries. The glomerular involvements may be seen as mesangiolysis with fibrin thrombi in the capillary loops. In chronic conditions, the glomerular capillary walls show double contours. In acute conditions, the injured vascular walls may show mucoid change with red blood cell fragments along with intimal proliferation of arteries and arterioles.

The five main pathological types of renal microvascular lesions in LN include 1) vascular immune complex deposits (ICD), 2) arteriosclerosis, 3) thrombotic microangiopathy, 4) non inflammatory necrotising vasculopathy, 5) true renal vasculitis. The contribution of the immune system dysfunction, especially the immune complex-induced vascular inflammation and antiphospholipid antibody associated thrombotic events along with activation and dysfunction of endothelial cells are the key mechanisms in the development of vascular lesions in LN. Recruitment of the immune cells such as T cells, monocytes and macrophages which induce platelet aggregation and microthrombus formation occurs due to alteration of the microvascular environment.[21]

TUBULOINTERSTITIAL LESIONS IN SLE

Tubular basement membranes may show granular deposits of immunoglobulins as well as complements which may be detected on immunofluorescence and electron microscopy. These deposits may not

be associated with interstitial inflammation which is seen predominantly as lymphocytes, monocytes and plasma cells i.e. interstitial inflammation is not directly correlated with the immune deposits. These deposits may cause active tubulitis. Tubular basement membrane deposits are more frequently associated with Class IV Lupus Nephritis but may be seen with any type of glomerular lesion. These deposits may also be seen in peritubular capillaries.

Tubular atrophy and interstitial fibrosis are associated with marked chronicity and are seen in severe glomerular diseases. These correlate with the degree of loss of GFR.[20]

LUPUS PODOCYTOPATHY

In some cases, widespread foot process effacement may be seen by Electron Microscopy. Rapid response to steroid therapy in the patients suggests that the podocyte lesions are more like minimal change disease and may not be associated with the consequence of immune deposits.[20]

ROLE OF DIRECT IMMUNOFLUORESCENCE

Direct immunofluorescence (DIF) is a crucial method for making precise diagnoses in renal pathology, particularly for diseases such as IgA nephropathy (IgAN), C1q nephropathy (C1qN), and C3 glomerulopathy (C3G), which cannot be diagnosed without it. The most commonly used IF technique is direct immunofluorescence.[22] The adjunctive technology of DIFM (Direct Immunofluorescence Microscopy) was first reported in 1980 by Sehgal et al. from the department of Immunopathology at PGIMER in India. The study documented the presence of immune complexes in post-mortem kidneys of patients with viral hepatitis.[23]

In lupus nephritis, pathologic findings may include evidence of immune deposits such as "full-house" staining for IgG, IgA, IgM, C1q, and C3, as well as strong C1q staining, as seen through tissue ANA tests and immunofluorescence. Additionally, electron microscopy may reveal extraglomerular immune deposits and tubuloreticular inclusions within

the endothelium. The combination of subendothelial and subepithelial deposits is also considered a hallmark of lupus nephritis.[24]

ROLE OF ELECTRON MICROSCOPY

Electron microscopy (EM) is a crucial tool in the diagnosis and management of lupus nephritis. It provides accurate characterization of the type of renal involvement and helps determine the degree of activity, which is important in guiding patient treatment. In some cases, EM may be the key to initial diagnosis of lupus nephritis in patients without diagnostic clinical manifestations or serologic markers. Additionally, EM evaluation plays a significant role in evaluating renal dysfunction in transplant patients and detecting the recurrence of lupus in the allograft. Some ultra-structural findings, when considered in the proper clinicopathologic context, can be highly suggestive or even diagnostic of lupus nephritis.[25]

Rasković S et al in their study in 2002 analyzed kidney biopsies of 58 patients with biopsy proven lupus nephritis and found that the histopathological findings of the renal biopsies belonged to class I lesion in 3.45% of patients, class IIA in 24.14%, class IIB in 31.03%, class III in 12.07%, class IV in 24.14%, class V in 3.35%, and class VI in 1.72% of cases according to the WHO classification. They also observed that the values of serum creatinine were elevated in 25.86% of patients, while creatinine clearance rate was lower (below 80 ml/min) in 75% of cases.[26]

In 2005, Rabbani MA et al in their study on kidney biopsies of 42 Lupus patients concluded that the main histological types as per WHO classification were class IV (n=27), class III (n=7) and class V (n=6). Immunofluorescence showed a predominantly granular pattern of IgG, IgA and C3. Renal manifestations included renal failure (50%), microscopic hematuria (67%), active urine sediment (22%), and proteinuria (74%). Proteinuria was in the nephrotic range in 45% cases.[27]

In the study by Satirapoj B et al in 2007, Class IV (61.5%) was the most common class on evaluation of renal histopathology. Crescentic

change and hyaline thrombi were found in 2.9% and 14.7% of biopsies, respectively. The most common manifestations of renal involvement in SLE patients were observed to be edema (75.0%). Urine sediment corresponding to a nephritic picture (defined as > 5 RBCs or any RBC casts per high power field in urine) and hypertension (blood pressure > 140/90 mmHg) were reported in 60.0% and 42.5% of the cases respectively. The mean value of proteinuria was 2,152.3 ± 1,620.2 mg/day.[28]

The renal biopsy specimens of the 61 patients included in a Greek study by Ourania D et al in 2011 revealed diffuse proliferative glomerulonephritis (class IV) as the most encountered type of renal disease in 23 patients, followed by the membranous lupus nephritis (class V) in 18 patients, the focal proliferative nephritis (class III) in 15 patients, and mesangial proliferative lupus nephritis (class II) in 5 patients. There were no differences regarding the gender or immunological profile and the histological class of nephritis identified on the biopsy samples.[29]

In 2016, a meeting of 18 members of an international nephropathology working group in Leiden, Netherlands was held where they proposed recommendations and adjustments in order to improve the clarity of definitions and classification of glomerular lesions. They provided new definitions for mesangial hypercellularity, cellular, fibrocellular and fibrous crescents. They also eliminated the class IV-S and IV-G subdivisions of class IV of lupus nephritis. They also proposed the inclusion of fibrinoid necrosis as a specific descriptor in the activity index.[30]

Rijnink EC et. al. in the year 2017, tried to correlate the prognosis of lupus nephritis patients with the histopathological features on kidney biopsy on the basis of EULAR criteria 2012. Their results showed that although ISN/RPS classes were poorly associated with the clinically relevant outcomes, prognostication in LN would benefit from the specific assessment of clinical variables and lesions currently obscured in the classification. The authors particularly suggested that an independent scoring should be done based on atleast fibrinoid

necrosis, fibrous crescents and IFTA to assess the risk of progressive renal dysfunction in addition to clinical findings.[31]

Aringer M et al. in 2019 developed a new classification criteria that was rigorously developed in 4 phases and jointly supported by European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR). The criteria for SLE included obligatory positive ANA at least once and a score from weighted addition of 7 clinical and 3 immunological (antiphospholipid antibodies, complement proteins, SLE-specific antibodies) domains between 2 to 10. Patients with greater than or equal to 10 points were classified. They found out that the new criteria had a sensitivity of 96.1% and specificity of 93.4% in diagnosing the disease, better than previous ACR 1997 criteria and Systemic Lupus International Collaborating Clinics 2012 criteria.[32]

In the study published in 2019 by Suda M et al on 100 Japanese patients, the researchers aimed to study the 2019 ACR/EULAR criteria in a real world setting. They concluded that the 2019 criteria for the classification of SLE achieved modestly high sensitivity in real-world practice. They possibly misclassified the real SLE cases as non-SLE, especially if patients had low titre (< 1:80) of ANA.[33]

Pons-Estel GJ et al studied the 2019 EULAR/ACR criteria on Latin American patients. The focus was on earlier patients' classification in comparison to 1982/1997 ACR or the 2012 SLICC criteria. Considering the 1982/1997 criteria as gold standard, the sensitivity was found to be 91.3% for 2019 EULAR/ACR criteria. This criteria allowed an earlier SLE patient classification in 7.4% (mean 0.67years) and 0.6% (mean 1.47 years) than the 1982/1997 ACR and the 2012 SLICC criteria, respectively. [34]

In a study done by Farah RI et al in 2019 that included a total of 79 patients resulted in Class IV being the most common pathological class of Lupus Nephritis (46.8%) followed by class V (19%) and class III (12.7%). They also found that there was a significant correlation between Class IV lupus nephritis and renal failure and Anti-dsDNA levels. The patients who developed ESRD were positive for anti-

dsDNA and hence they concluded that anti DNA positivity was related with disease severity.[35]

In a study done by Chelliah V et al on 50 patients, the serological profile of SLE showed a significant correlation with histopathology of lupus nephritis. Anti-dsDNA, low C3, and low C4 had a significant independent correlation ($p<0.05$) with proliferative LN (class IV, IV and V). Of 50 patients, class IV lupus nephritis was seen in 35 (70%), class II in 7 (14%) , class V in 4(8%) and 4 patients (8%) had class IV and V on renal biopsy. The prevalence of anti-dsDNA was 97.1% in LN and 97.4% (38 of 39 pts) in proliferative LN ($p<0.001$). The C3 level was low in 68% of patients with LN and 84.6% with proliferative LN ($p <0.001$). C4 level was low in 74% of patients with LN and 87.2% with proliferative LN ($p <0.001$). In their study, 72% (28 of 39 pts) of the patients with proliferative LN (class IV, IV and V) had the combination of anti-dsDNA positivity, low C3 and low C4 levels but none of the patients with class II or class V LN had this combination of serology.[36]

Hashmi AA et al. in 2020 evaluated renal biopsies of 128 patients that fulfilled the clinical and serological criteria of LN. They observed that the mean age was 28.85±12.24 years with most of the patients belonging to the age group ≤25 years (48.4%). Class IV was the most common class on renal biopsy i.e. (46.9%). Active lesions were seen in 66 (51.6%) patients, with endocapillary hypercellularity being the most common active lesion type, i.e. 81.8%. Chronic lesions were noted in 42 (32.8%) patients, with glomerular sclerosis being the most common chronic lesion type, i.e. 69%. Females were more likely to present at advanced lupus stage compared to males, and older patients (>50 years) had a higher chance to present at a late stage (class IV and higher). Active lesions were significantly found more frequently in classes III and IV, while chronic lesions were more likely to present in classes III to V. [37]

In 2022, in a study done by Javeed S et al. on 58 renal biopsies, the male-to-female ratio was observed to be 1:6.5 with the mean age 23.09±9.23 years. Serum urea levels were found to be increased in 36 (62.10%) patients while serum creatinine levels were found to be

increased in 43 (74.12%) patients. Nephritic range proteinuria was seen in 14 (24.10%) patients, while 44 (75.90%) patients had proteinuria in the nephrotic range. Positivity for Anti-double stranded DNA antibody was seen in 49 (84.50%) patients. Microscopic hematuria was present in 46 (79.30%) patients. Majority of the patients belong to class V, 25 (43.10%), followed by class IV, 16 (27.59%). Full-house IF pattern was seen in the majority of patients.[38]

MATERIALS AND METHODS

SOURCE OF DATA

This study was conducted for a period of 2 years, one year retrospective (1st March 2020-28th Feb 2021) and one year prospective (1st March 2021- 28th Feb 2022), in the department of Pathology, Dayanand Medical College and Hospital Ludhiana. All the kidney biopsies of clinically diagnosed SLE patients included in the study were analyzed with the reference to the light microscopic findings and direct immunofluorescence. Correlation with ELISA, immunoblot and indirect immunofluorescence findings was done wherever possible.

METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA

Kidney biopsies from clinically diagnosed SLE patients

EXCLUSION CRITERIA

All the kidney biopsies of Non SLE patients

METHODOLOGY

All kidney biopsies satisfying the inclusion criteria received in the Department of Pathology over a period of 2 years were analyzed with reference to light microscopic and direct immunofluorescence findings and were correlated with the clinical profile i.e. clinical presentation, diagnosis and laboratory parameters. All the biopsies were histologically analyzed and typed based on light microscopy findings using routine (H&E) and special stains. Correlation with immunofluorescence findings was done whenever possible.

Renal biopsy specimens received were fixed in 10% formalin over a period of 24-48 hours and then processed for paraffin embedding. Sections for light microscopy were stained with Haematoxylin-Eosin, Periodic Acid-Schiff, Masson's Trichrome and Methenamine Silver stain. Special stains such as congo red were done wherever required. The slides were then subjected to the histopathological examination under both low power (100X) and high power (400X). Step sections

were done whenever required. Interpretation of histopathological findings was done.

2018 modification of ISN/RPS 2003 classification was used to further categorize these cases based on morphological and direct immunofluorescence findings.

This classification included the following classes-

- Class I-Minimal mesangial lupus nephritis
- Class II-Mesangial proliferative lupus nephritis
- Class III-Focal lupus nephritis
- Class IV-Diffuse lupus nephritis
- Class V-Membranous lupus nephritis
- Class VI-Advanced sclerosing lupus nephritis.

Histologically, the kidney biopsies that showed normal glomeruli by light microscopy with presence of mesangial immune deposits on direct immunofluorescence were classified as Class I lupus nephritis.

The kidney biopsies containing mesangial proliferative lesions seen as mesangial hypercellularity of any degree or mesangial matrix expansion along with mesangial deposits on direct immunofluorescence were classified as Class II lupus nephritis.

Kidney biopsies with presence of focal proliferative, sclerotic or necrotising lesions were classified as Class III lupus nephritis. Focal involvement refers to involvement of less than half of the glomeruli available for microscopic examination.

The biopsies with presence of diffuse proliferative, necrotising or sclerotic lesions were classified as Class IV lupus nephritis. Diffuse involvement refers to involvement of more than 50% of glomeruli available for microscopic examination.

Histologically, the kidney biopsies showing presence of variable mesangial expansion with diffuse thickening of the glomerular capillary walls along with granular deposits of immunoglobulins and complements along the glomerular capillary walls and in the mesangium on direct immunofluorescence were classified as Class V lupus nephritis.

The kidney biopsies with presence of >90% glomeruli having global or extensive segmental sclerosis without residual activity were classified as Class VI lupus nephritis.

Paraffin block tissue processing:

The tissue fixed in 10% formalin was processed through a series of solutions in the automatic tissue processor as follows:

- Acid formalin for 4 hours
- Formal alcohol for 1 hour
- 80% ethyl alcohol for 1 hour
- 90% ethyl alcohol for 1 hour
- Absolute ethyl alcohol for 1 hour
- Acetone (1st change) for 1 hour
- Acetone (2nd change) for 1 hour
- Xylene (1st change) for 1 hour
- Xylene (2nd change) for 1 hour
- Paraffin (1st change at 58-60°C) for 1 hour
- Paraffin (2nd change at 58-69°C) for 1 hour

The paraffin embedded tissue blocks were cut at 2-3 microns thickness by rotary microtome. These sections were then transferred onto albuminized slides. These sections were labeled properly with the help of glass marking pencils.

Procedure of Hematoxylin and Eosin Staining

1. Slides were kept on a hot plate in order to dewax the sections at temperature up to 60°C.
2. Then slides were deparaffinized in xylene.
3. Then hydration was done by passing through descending rates of alcohol to water.
4. The slides were then stained in Harris Hematoxylin for 15 minutes. Sections were washed well in running tap water until sections turned blue. These sections were then differentiated in 1% acid alcohol (1% HCI in 70% alcohol) for 5-10 seconds, washed well in tap water till sections were again blue for 5 minutes or less.

5. These were again stained in 1% Eosin for 10 min followed by washing in running tap water for 1-5 minutes.
6. Sections were dehydrated once again through ascending grades of Alcohol, cleaned in Xylene and mounted with DPX (distyrenedibutylphthalate xylene) avoiding air bubbles.
7. Stained slides were then subjected to microscopic examination under both low (100x) and high power (400x).

Procedure for Periodic Acid Schiff staining (P.A.S.)

1. Slides were washed with distilled water.
2. Then slides were kept in periodic acid solution for 5 minutes.
3. Then slides were washed with water.
4. Then the slides were kept in Schiff reagent for 10-20 minutes.
5. Then slides were washed again with water.
6. Then the slides were counter stained very lightly with Harris hematoxylin (1 dip).
7. These slides were then differentiated with 1% acid alcohol.
8. The slides were then washed with running tap water for 5 minutes.
9. Then the slides were mounted with DPX.
10. Slides were then examined under a light microscope.

Procedure for Methenamine Silver stain

1. The slides were washed under water.
2. Then the slides were put in 1% chromic acid for 1 hour.
3. Then they were again washed with tap water.
4. After washing silver methenamine stain was put on the slides.
5. After staining, slides were kept at 60° C for one hour till the sections become brown.
6. Then the slides were toned with 1% gold chloride.
7. Then the slides were counter stained with light green 1%.
8. After counter staining, slides were dehydrated, cleared and mounted in DPX.

Procedure for Mason's trichrome stain

1. The slides were washed under water.
2. Mercury pigment was removed by iodine (iodine bleach with sodium

thiosulphate for 5 minutes).

3. Slides were washed in tap water again.
4. Nuclei were stained by Celestin blue hematoxylin for 5 min,
5. Differentiated with 1% acid alcohol.
6. Washed in tap water.
7. Stain with acid fuschin for 5 minutes.
8. Rinsed in water.
9. Treated with phosphomolybdic acid for 5 minutes.
10. Slides were then drained.
11. Slides were then stained with methyl blue for 2-5 minutes.
12. Rinsed in water.
13. Treated with 1% acetic acid for 2 minutes.
14. Dehydrated through alcohol & clear and then mounted in DPX.

DIRECT IMMUNOFLUORESCENCE

1. Blocks were prepared in embedding media and kept in the cryostat for 30 minutes at -20 degree centigrade.
2. Two to three microns thin sections were cut and put on the plain slides.
3. All slides were kept in a deep freezer at -20 degree centigrade overnight.
4. Next day the slides were taken out from the deep freezer and left at room temperature for one hour.
5. Antibodies were diluted in phosphate buffer saline (PBS).
6. Microcentrifuge for 3 minutes at full speed.
7. Antibodies were dispensed (FITC conjugated antisera specific for IgG, IgA, IgM, C3 and fibrinogen) covering the whole slide.
8. All slides were placed in a moist chamber and kept in an incubator for 1 hour at 37 degree centigrade.
9. Slides were taken out from the incubator and washed in PBS buffer at least 3 times
10. Slides were mounted with buffered glycerol
11. Stored in the dark.
12. Examined under fluorescent microscope filled with ultraviolet light source under ideal excitation and barrier filter combination to visualize-

a) Presence or absence of immunoreactants
b) Site of deposition of immunoreactants
c) Pattern of immunofluorescence(linear/granular/both)

Electron microscopy was not performed in our study as the facility is not available in our department.

OBSERVATIONS AND RESULTS

The present 2 year study, 1 year retrospective [1st March 2020 to 28th Feb 2021] and 1 year prospective [1st March 2021 to 28th Feb 2022] on Morphological Spectrum of Lupus Nephritis was conducted in the Department of Pathology, Dayanand Medical College & Hospital, Ludhiana. A total of 415 renal biopsies were received during this time period, out of which 27 were received with a clinical suspicion of lupus nephritis based upon clinical signs and symptoms and ANA positivity on indirect immunofluorescence. These cases were further analyzed in detail based on microscopic examination and direct immunofluorescence findings with regards to class, severity, activity and chronicity and were further classified into six classes according to 2018 modification of ISN/RPS 2003 classification.

All the patients were positive for ANA on indirect immunofluorescence at a titre of >1:80 on Hep-2 cells.

Table 3. Distribution of cases according to age (n=27)

Age	No of cases	Percentage
1-20	2	7.4
21-40	16	59.3
41-60	6	22.2
61-80	3	11.1

Out of the 27 cases studied, majority of the patients belonged to the age group 21-40 years (59.3%) followed by 22.2% patients in the age group 41-60 years. There were 11.1% patients in the age group 61-80 years and the least number of patients (7.4%) were less than 20 years of age. The youngest patient was 7 years old while the eldest was 73 years old with a mean age of 38.26±14.5 years. (Table 3)

Table 4. Distribution of cases according to gender (n=27)

Gender	No of Cases	Percentage
Male	5	18.5
Female	22	81.5

Females were more commonly affected with a Male to Female ratio of 1:4.4 as depicted in table 4.

Table 5. Distribution of cases according to 2018 modification of ISN/RPS 2003 classification (n=27)

ISN/RPS class	No of cases	Percentage
I	0	0
II	2	7.4
III	5	18.5
IV	13	48.2
V	3	11.1
VI	1	3.7
Mixed V+III	3	11.1
Mixed V+IV	0	0
Total	27	100

According to the 2018 modification of ISN/RPS 2003 classification system, almost half of our cases i.e. 13 cases (48.2%) were found to have Diffuse Lupus Nephritis i.e. Class IV LN. The second most common type of lupus nephritis on biopsy was Focal Lupus Nephritis i.e. Class III LN with 5 cases (18.5%). Three cases were classified as Class V LN i.e. membranous lupus nephritis (11.1%). Two cases were classified as Class II LN i.e. mesangial proliferative lupus nephritis (7.4%) and 1 was classified as Class VI LN i.e. advanced sclerosing lupus nephritis (3.7%). Three cases (11.1%) were of the mixed morphological pattern where both of them belonged to Class III+V while none of our cases were diagnosed as Class IV+V or class I LN.

Table 6. Spectrum of clinical presentation (n=27)

Clinical presentations	No of cases	Percentage
Synovitis	16	59.2
Fever	12	44.4
Pedal/ generalized edema	12	44.4
Nonscarring alopecia	11	40.7
Acute Cutaneous Lupus	10	37.0
Oral/Nasal ulcers	8	29.6
Serositis	5	18.5
Frothy urine	5	18.5
Gross Hematuria	3	11.1
Shortness of breath	3	11.1
Pain abdomen	3	11.1
Neurological	2	7.4
Oliguria	2	7.4
Chronic Cutaneous Lupus	0	0

All the patients diagnosed with SLE suffered from multiple signs and symptoms. The most common clinical complaint was synovitis in 16 patients (59.2%) followed by fever and oedema in 12 patients (44.4%), non-scarring alopecia in 11 patients (40.7%) and malar rash in 10 patients (37%). Other symptoms including frothy urine, pain in the abdomen, hematuria, shortness of breath and oliguria were also observed. (Table 6).

Table 7: Class-wise distribution of cases based on clinical presentation (n=27)

Clinical presentations	Class II (n=2)	Class III (n=5)	Class IV (n=13)	Class V (n=3)	Class VI (n=1)	Class III+V (n=3)
Synovitis	2(100%)	3(60%)	7(53.8%)	1(33.3%)	1(100%)	2(66.7%)
Fever	1(50%)	3(60%)	6(46.1%)	1(33.3%)	0(0%)	1(33.3%)
Pedal/ generalized edema	0(0%)	1(20%)	9(69.2%)	1(33.3%)	0(0%)	1(33.3%)
Nonscarring alopecia	1(50%)	2(40%)	5(38.4%)	2(66.7%)	0(0%)	1(33.3%)
Acute Cutaneous Lupus	0(0%)	3(60%)	2(15.3%)	2(66.7%)	1(100%)	2(66.7%)
Oral/Nasal ulcers	2(100%)	1(20%)	4(30.7%)	1(33.3%)	0(0%)	0(0%)
Serositis	0(0%)	1(20%)	3(23.0%)	1(33.3%)	0(0%)	0(0%)

Frothy urine	0(0%)	3(60%)	0(0%)	1(33.3%)	0(0%)	1(33.3%)
Gross Hematuria	0(0%)	0(0%)	2(15.3%)	0(0%)	0(0%)	1(33.3%)
Shortness of breath	0(0%)	2(40%)	0(0%)	1(33.3%)	0(0%)	0(0%)
Pain abdomen	0(0%)	1(20%)	2(15.3%)	0(0%)	0(0%)	0(0%)
Neurological	1(50%)	0(0%)	1(7.6%)	0(0%)	0(0%)	0(0%)
Oliguria	0(0%)	1(20%)	0(0%)	1(33.3%)	0(0%)	0(0%)
Chronic Cutaneous Lupus	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Majority of the clinical symptoms such as synovitis, fever, pedal edema, non-scarring alopecia, oral/nasal ulcers, serositis and pain in abdomen were more commonly seen in patients classified as class IV lupus nephritis on kidney biopsy while acute cutaneous lupus and frothy urine were more commonly seen in patients classified as class III lupus nephritis. None of the patients presented with a chief complaint of chronic cutaneous lupus as shown in Table 7.

DISTRIBUTION OF CASES BASED ON HEMATOLOGICAL AND SEROLOGICAL PARAMETERS

Table 8. Distribution of cases based on severity of anemia (n=27)

Hemoglobin g/dl	No of cases	Percentage
Severe (<8)	5	18.5
Moderate (8-10)	12	44.5
Mild (10-12)	8	29.6
Normal	2	7.4
Total	27	100

Anemia was categorized as mild, moderate and severe based on the hemoglobin levels. Patients with hemoglobin levels between 10-12 g/dl, 8-10 g/dl and less than 8 were categorized as mild, moderate and severe anemia respectively.

On evaluation of the blood counts, among the 27 patients in our study, 12 patients (44.5%) were moderately anemic followed by 8 patients (29.6%) having mild degree of anemia while 5 (18.5%) of them were severely anemic. Only 2 (7.4%) patients had hemoglobin levels within normal limits. (Table 8)

Table 9: Class-wise distribution of cases based on hemoglobin levels (n=27)

Haemoglobin g/dl	Class II (n=2)	Class III (n=5)	Class IV (n=13)	Class V (n=3)	Class VI (n=1)	Class III+V (n=3)
Severe (<8)	0 (0%)	1 (20%)	3 (23.0%)	0 (0%)	0 (0%)	1 (33.3%)
Moderate (8-10)	1(50%)	1 (20%)	7 (53.8%)	1 (33.3%)	1 (100%)	1 (33.3%)
Mild (10-12)	1 (50%)	3 (60%)	3 (23.0%)	1 (33.3%)	0 (0%)	1 (33.4%)
Normal	0 (0%)	0 (0%)	0 (0%)	1 (33.4%)	0 (0%)	0 (0%)

Severe anemia was more commonly seen in patients classified as class IV lupus nephritis i.e. out of the 5 patients suffering from severe anemia, 3 were classified as class IV lupus nephritis on kidney biopsy while the other two belonged to class III lupus nephritis and class III+V lupus nephritis each. (Table 9)

Table 10. Distribution of cases according to total leucocyte count (n=27)

TLC (x10^3/uL)	No of cases	Percentage
<4	5	18.5
4-11	18	66.7
>11	4	14.8
Total	27	100

Leucopenia was observed in 5 cases (18.5%) and 4 cases (14.8%) had leucocytosis with total leucocyte count (TLC) of more than 11x10^3/uL while the rest of them had total leucocyte count within normal limits. Out of the 5 cases with leucopenia, 3 of them belonged to class IV lupus nephritis. (Table 10)

Table 11. Distribution of cases according to platelet count (n=27)

Platelets (x10^3/uL)	No of cases	Percentage
<50,000	0	0
50000-100000	5	18.6
100000-150000	3	11.1
>150000	19	70.3
Total	27	100

Five cases (18.6%) were observed to have platelet counts between 50000-100000/uL, three cases (11.1%) had platelet counts between 100000-150000/uL while rest of the cases had platelet counts of more than 150000/uL, however, none of the patients suffered from severe thrombocytopenia. (Table 11)

Table 12. Class-wise distribution of cases based on hematological parameters (n=27)

Class	Anaemia	Bicytopenia	Pancytopenia	Normal
Class II (n=2)	-	1	1	-
Class III (n=5)	4	1	-	-
Class IV (n=13)	9	3	-	1
Class V (n=3)	2	1	-	-
Class VI (n=1)	1	-	-	-
Class III+V (n=3)	3	-	-	-
Total	19	6	1	1

Isolated anaemia was the most common presentation among the hematological parameters in our study and was observed in 19 out of 27 cases which was followed by bicytopenia in 6 cases. Pancytopenia was seen in only 1 case and 1 case had normal hematological parameters. Nine out of nineteen patients (47.3%) with isolated anemia were diagnosed as class IV LN. Three out of 6 cases (50%) of bicytopenia belonged to class IV LN. The only case with pancytopenia belonged to class II LN. (Table 12)

Table 13. Class-wise distribution of cases with respect to hematuria, proteinuria and serum creatinine levels (n=27)

	Hematuria		Proteinuria		Serum creatinine	
Class	Present (n=23)	Absent (n=4)	Nephrotic range (n=14)	Subnephrotic range (n=13)	Normal (n=14)	Raised (n=13)
Class II (n=2)	2 (100%)	-	1 (50%)	1 (50%)	2(100%)	0
Class III (n=5)	4 (80%)	1 (20%)	3 (60%)	2 (40%)	4 (80%)	1 (20%)
Class IV (n=13)	12 (92.3%)	1 (7.7%)	7 (53.8%)	6 (46.2%)	6 (46.2%)	7 (53.8%)
Class V (n=3)	1 (33.3%)	2(66.7%)	2 (66.7%)	1 (33.3%)	1 (33.3%)	2 (66.7%)

Class VI (n=1)	1 (100%)	-	-	1 (100%)	0	1 (100%)
Class III+V (n=3)	3 (100%)	-	1 (33.3%)	2 (66.7%)	1 (33.3%)	2 (66.7%)

Nephrotic syndrome is characterized by proteinuria (nephrotic range->3.5g/24 hr), hypoalbuminemia, generalized edema, hyperlipidemia and lipiduria. Nephritic syndrome is characterized by inflammation in the glomeruli and is diagnosed when a patient has hematuria, proteinuria (subnephrotic range-<3.5g/24 hr), azotemia and hypertension. In our study of 27 patients, 14 patients (51.8%) presented with sub-nephrotic range proteinuria (<3.5g/24hr) while 13 patients presented with nephrotic range proteinuria (>3.5g/24hr). Nephrotic range and sub-nephrotic range proteinuria each were observed in 50% of the cases of class II. Classes III, IV and V had more percentage of cases with sub-nephrotic range proteinuria while class III+V had more percentage of cases with nephrotic range proteinuria. However, the single case of class VI LN had nephrotic range proteinuria.(Table 13)

Serum creatinine levels were raised in 13 cases (48.1%) with 5 of these cases (18.6%) having serum creatinine levels more than 3 mg/dl. Raised serum creatinine levels were seen in 20% cases of class III, 53.8% cases of class IV, 66.7% cases of class V and III+V each and 100% cases of class VI LN. (Table 13)

Microscopic hematuria detected by the Dipstick method was evident in 23 cases (85.1%). Of the four cases without microscopic hematuria two (50%) belonged to class V LN as depicted in table 13.

Table 14. Frequency of autoantibodies in the patients (n=27)

Antibodies	Positive	Negative
Anti-dsDNA	19 (70.3%)	8 (29.7%)
Anti Smith	13 (48.1%)	14 (51.9%)
Anti Nucleosome	14 (51.8%)	13 (48.2%)
Anti Histone	12 (44.4%)	15 (55.6%)
Anti SSA	9 (33.3%)	18 (66.7%)
Anti SSB	8 (29.6%)	19 (70.4%)
Anti Scl 70	0	27 (100%)

All the cases in our study were positive for ANA by indirect immunofluorescence. High-titer IgG antibodies to double-stranded DNA (dsDNA) are specific for SLE. Of the 27 cases in our study, 19 cases (70.4%) had positive anti-dsDNA antibodies by ELISA. Among the total 27 cases, 13 cases (48.1%) showed positivity for antiSmith antibodies. Out of these positive cases, 4 cases had strong positivity and 1 case had weak positivity for antiSmith antibodies. 14 cases (51.8%) showed positivity for anti-nucleosome antibodies while 12 cases (44.4%) showed positivity for anti-histone antibodies. Anti Ro and anti La antibodies were positive in 33.3% and 29.6% cases respectively while Anti Scl 70 antibodies were not positive in any case as depicted in table 14.

Table 15. Distribution of cases based on serum complement levels (n=27)

Complement	Reduced	Normal
C3	21(77.7%)	6 (22.3%)
C4	16 (59.2%)	11 (40.8%)

Table 16. Class-wise distribution of cases based on serum complement levels (n=27)

Complement	Class II (n=2)	Class III (n=5)	Class IV (n=13)	Class V (n=3)	Class VI (n=1)	Class III+V (n=3)
Reduced C3	1(50%)	5(100%)	11(84.6%)	2(66.7%)	-	2(66.7%)
Reduced C4	1(50%)	4(80%)	8(61.5%)	2(66.7%)	-	1(33.3%)

Complement activation plays a key role in the pathophysiology of lupus nephritis. Hence, it is important to monitor the serum complement levels in order to monitor the disease activity. Among the total 27 cases in our study, C3 levels were observed to be reduced in 21 cases (77.7%) while serum C4 levels were reduced in 16 cases (59.2%). Only one case was observed to have isolated low C4 levels with normal C3 levels and was classified as class V LN. In Class II, 50% of the cases had reduced C3 levels, while 50% had reduced C4 levels. In Class III, 100% of the cases had reduced C3 levels and 80% had reduced C4 levels. In Class IV, 84.6% of the cases had reduced C3 levels and 61.5% had reduced C4 levels. For Class V, 66.7% of the cases had reduced C3 and C4 levels. In Class VI, there were no cases with reduced C3 or C4 levels. In Class III+V, 66.7% of the individuals had reduced C3 levels and 33.3% had reduced C4 levels.(Table 15 & 16)

Table 17 Distribution of cases according to the IF positivity (n=25)

Immunofluorescence deposits	No of cases (n=25)	Percentage
IgG	25	100
C3c	23	92
C1q	22	88
IgM	20	80
IgA	20	80

Table 18. Class-wise distribution of cases based on IF positivity (n=25)

	IgG	IgA	IgM	C3c	C1q
Class II (n=2)	2	2	2	2	2
Class III (n=4)	4	3	4	4	4
Class IV (n=13)	13	11	10	12	11
Class V (n=3)	3	3	3	3	3
Class III+V (n=3)	3	1	1	2	2

Out of the 27 cases studied, 18 cases (66.6%) showed full house positivity on direct immunofluorescence that is these cases showed deposits of IgG, IgA, IgM, C1q and C3c while one case which was categorized as advanced sclerosing lupus nephritis i.e. Class VI had no deposits of immunoglobulins in the glomeruli. Renal biopsy for direct immunofluorescence in one case that was diagnosed as class III LN on light microscopy, was inadequate for opinion. IgG was seen in all the cases (100%) having adequate glomeruli except one case which had sclerosed glomeruli. This was followed by C3c in 23 cases (92%) and C1q in 22 cases (88%). Individual positivity of each immunoglobulin is highlighted in table 17 and 18.

Table 19. Class wise distribution of the various histological parameters (n=27)

Histopathology findings	Class II (n=2)	Class III (n=5)	Class IV (n=13)	Class V (n=3)	Class VI (n=1)	Class III+V (n=3)
Mesangial hypercellularity	2 (100%)	5 (100%)	13 (100%)	3 (100%)	0	1(33.3%)
Endocapillary proliferation	0	4 (80%)	10 (76.9%)	0	0	2 (66.6%)
Leucocytic infiltrate/Karyorrhexis	0	5 (100%)	13 (100%)	0	0	2 (66.6%)
Wireloop deposits	0	0	2 (15.3%)	0	0	0
Fibrinoid necrosis	0	1 (20%)	5 (38.4%)	0	0	0
Cellular Crescents	0	2 (40%)	11 (84.6)	0	0	1 (33.3%)
Interstitial inflammation	0	3 (60%)	12 (92.3%)	2 (66.6%)	1 (100%)	1 (33.3%)

Fibrous crescent	0	0	2 (15.3%)	0	0	1 (33.3%)
Glomerular sclerosis	0	0	4 (30.7%)	0	1 (100%)	0
Tubular atrophy	0	4 (80%)	8 (61.5%)	2 (66.6%)	1 (100%)	1 (33.3%)
Basement membrane thickening	0	2 (40%)	6 (46.1%)	3 (100%)	0	3 (100%)

Table 19 shows the distribution of various histological parameters across different classes of lupus nephritis. The parameters include mesangial hypercellularity, endocapillary proliferation, leucocytic infiltrate/karyorrhexis, wireloop deposits, fibrinoid necrosis, cellular crescents, interstitial inflammation, fibrous crescent, glomerular sclerosis, tubular atrophy and basement membrane thickening. All classes (II-V) showed 100% mesangial hypercellularity. Endocapillary proliferation was seen in 80% of class III cases, 76.9% of class IV cases and 66.6% cases of class III+V LN. Leucocytic infiltrate/karyorrhexis was seen in 100% of class III, IV, VI and 66.6% cases of class III+V LN cases. Wireloop deposits were seen in 15.3% of class IV cases. Fibrinoid necrosis was seen in 20% of class III cases and 38.4% of class IV cases. Cellular crescents were seen in 40% of class III cases and 84.6% of class IV cases. Interstitial inflammation was seen in 60% of class III cases and 92.3% of class IV cases. Fibrous crescent was seen in 15.3% of class IV cases and 33.3% cases of class III+V. Glomerular sclerosis was seen in 30.7% of class IV cases and 100% of class VI cases. Tubular atrophy was seen in 80% of class III cases, 61.5% of class IV cases, and 66.6% of class V cases. Basement membrane thickening was seen in 40% of class III cases, 46.1% of class IV cases, and 100% of class V and III+V cases.

Table 20: Class-wise distribution of active and chronic lesions (n=27)

Class	Active lesions		Chronic lesions	
	No of cases	Percentage	No of cases	Percentage
Class II (n=2)	0	0	0	0
Class III (n=5)	5	100	1	20
Class IV (n=13)	13	100	9	69.2
Class V (n=3)	0	0	2	66.7
Class VI (n=1)	0	0	1	100
Class III+V (n=3)	3	100	1	33.3

Active lesions include endocapillary hypercellularity, extracapillary proliferation, fibrinoid necrosis, leucocytic infiltration, karyorrhexis and wire-loop deposits while chronic lesions include glomerular sclerosis, interstitial fibrosis, tubular atrophy and fibrous crescents. In our study, active lesions were more commonly seen in class III, IV and III+V lupus nephritis while chronic lesions were most commonly observed in class IV and V lupus nephritis as shown in table 20.

MODIFIED NIH ACTIVITY INDEX(0-24)	SCORE
Endocapillary Hypercellularity	0-3
Neutrophils/Karyorrhexis	0-3
Fibrinoid Necrosis	(0-3)x2
Hyaline deposits	0-3
Cellular/fibrocellular Crescents	(0-3)x2
Interstitial Inflammation	0-3
Total	0-24

Modified Activity score according to 2018 modification of ISN/RPS 2003 classification is based on endocapillary hypercellularity, neutrophilic infiltration/ karyorrhexis, fibrinoid necrosis, hyaline deposits, cellular/fibrocellular crescents and interstitial inflammation. A score of 0-3 was given for each entity except fibrinoid necrosis and cellular/fibrocellular crescents where the score was multiplied by 2. The score was based on the number of glomeruli involved i.e. <25% glomeruli involved (1+), 25-50% glomeruli involved (2+), >50% glomeruli involved (3+).

Table 21. Distribution of cases based on modified activity score (n=27)

Modified activity index	No of cases	Percentage
0-12	24	88.8
12-24	3	11.1

Modified activity score of more than 12 is associated with a poorer prognosis. Of all the cases in our study, an activity score of more than 12 was observed in 3 cases (11.1%) while the rest of the cases had an activity score of <12.

Table 22. Mean activity and chronicity indices of various classes (n=27)

Class	Mean activity index	Mean chronicity index
Class II (n=2)	0.00	0.00
Class III (n=5)	5.00±2.55	1.80±2.17
Class IV (n=13)	8.69±3.66	2.6±1.85
Class V (n=3)	2.00±2.65	0.67±1.15
Class VI (n=1)	2.00	9.00
Class III+V (n=3)	2.67±1.15	3.6±5.51

The highest mean activity index was seen in class IV and class III as 8.69±3.66 and 5.00±2.55 respectively while the highest mean chronicity index was seen in class VI which was 9 followed by class III+V and then class IV.

Anti neutrophil cytoplasmic antibody (ANCA) serology was available in 8 out of 27 cases in our study. Of the eight cases, only 2 cases were positive for ANCA with both of them having anti-myeloperoxidase antibodies. One of these cases belonged to class IV LN and the other one belonged to class III+V LN.

CLASS I LUPUS NEPHRITIS (MINIMAL MESANGIAL LUPUS NEPHRITIS) (n=0)

None of the cases in our study were diagnosed as Class I Lupus Nephritis

CLASS II LUPUS NEPHRITIS (MESANGIAL PROLIFERATIVE LUPUS NEPHRITIS) (n=2)

Both the cases presented with oral/nasal ulcers and synovitis along with other clinical symptoms that included periorbital swelling and reduced appetite. One of these patients had neurological symptoms - altered sensorium.

On evaluation of the blood counts, both the cases of Class II LN had mild anemia with hemoglobin levels within 10-12 g/dl and leucopenia and one case with moderate degree of thrombocytopenia with platelets count of 80000/uL. Serum creatinine levels were within the normal range in both the cases.

Morphologically, renal biopsy in both the cases showed mesangial hypercellularity with mesangial matrix expansion. However, no proliferative/sclerosing/necrotizing lesions were evident. No significant interstitial inflammation was seen in both the cases.

In one case the small arteries showed thickening of the vessel wall with mild medial hypertrophy while blood vessels were morphologically unremarkable in the other case. Tubules were unremarkable in both the cases.

None of the cases of class II showed IFTA (Interstitial fibrosis and Tubular Atrophy) more than 10%. Both the cases had a Modified Activity Index of 0/24 and Modified chronicity index of 0/12.

On direct immunofluorescence full house positivity was seen in both the cases.

CLASS III LUPUS NEPHRITIS (FOCAL LUPUS NEPHRITIS), (n=5)

Five cases were diagnosed as class III LN on biopsy. The most common age group was 21-40 years [3 cases (60%)]. Females were more commonly affected with male to female ratio being 1:4.

Table 23. Distribution of class III cases according to gender (n=5)

Gender	No of Cases	Percentage
Male	1	20
Female	4	80

The most common clinical presentation of Class III cases included malar rash, synovitis and fever each in 3 cases (60%), non-scarring alopecia in 2 cases (40%). Pedal edema and serositis were seen in 1 case each. Other symptoms included frothy urine in 3 cases (60%), shortness of breath in 2 cases (40%) and pain abdomen and oliguria in 1 case each.

On examination of the blood counts, 3 patients had anemia of mild degree that is hemoglobin levels between 10-12 g/dl while 1 case had moderate degree of anemia with hemoglobin level of 9.4 g/dl and one case had severe anemia with hemoglobin level of 7.7 g/dl. Four cases had TLC levels within normal limits while one presented with leucocytosis with total leucocyte count of $19.8x10^3$/uL. Platelet counts were within normal limits in 4 cases i.e. more than 150000/uL. Only one case had platelet count of less than 150000/uL i.e. 116000/uL.
Four cases (80%) had normal serum creatinine levels and only 1 (20%) had raised serum creatinine levels i.e. 1.64 mg/dl.

Table 24. Distribution of class III cases according to morphological features (n=5)

	Morphological feature	No of cases	Percentage
Glomerular lesions	Focal involvement (<50% glomeruli involved)	5	100
	Diffuse involvement (>50% glomeruli involved)	0	0
	Mesangial hypercellularity	5	100
	Endocapillary hypercellularity	4	80
	Leukocyte infiltration/karyorrhexis	5	100
	Crescents	2	40
	Basement membrane thickening	2	40
	Fibrinoid necrosis	1	20
	Wire Loop deposits	0	0
	Hyaline deposits	0	0

Tubules	Tubular atrophy	4	80
Interstitium	Interstitial inflammation	3	60
	Interstitial fibrosis	1	20

Morphologically, the kidney biopsies of all of these cases showed mesangial hypercellularity with neutrophilic infiltration. Proliferative lesions were seen in less than 50% of the glomeruli examined in all the cases. Endocapillary hypercellularity was seen in 4 cases (80%) with two of these cases showing presence of cellular crescents. One case (20%) showed presence of fibrinoid necrosis. Irregular basement membrane thickening was identified in 2 cases. Among the chronicity features, 2 (40%) cases showed glomerular sclerosis, interstitial fibrosis was seen in 1 case (20%) and tubular atrophy in 4 cases (80%).

Lymphomononuclear infiltrate was evident in 2 cases, mixed inflammatory infiltrate was seen in 2 cases while one case showed no significant inflammatory infiltrate.

Degenerative changes of the tubules seen as loss of brush border epithelium was seen in 4 cases with one of them showing presence of waxy and dense casts. Tubules were unremarkable in one case. Vascular changes were observed in only one out of the five cases seen as mild medial thickening. Only one case showed 30% IFTA on morphology. (Table 24&25)

All of these cases had the Modified Activity Index of less than 12. Modified chronicity index of 5/12 was seen in one case, 3/12 was seen in one case and 1/12 was seen in one case. The remaining 2 cases had a modified chronicity index of 0/12.

Table 25. Distribution of class III cases based on IFTA (n=5)

IFTA%	No of cases	Percentage
<5%	3	60
6-25%	1	20
26-50%	1	20
>50%	0	0

Among the cases diagnosed as Class III lupus nephritis, 3 cases showed full house positivity on direct immunofluorescence. One case showed positivity for IgG, IgM, C3c and C1q while renal biopsy in the other case was inadequate for opinion.

CLASS IV LUPUS NEPHRITIS (DIFFUSE LUPUS NEPHRITIS) (n=13)

It is characterized by proliferative, necrotising or sclerotic lesions involving more than 50% of glomeruli.

Among the cases diagnosed as class IV lupus nephritis, the most common age group was 21-40 years with 8 cases (61.5%). 3 cases (23.1%) belonged to the age group 41-60 years and 2 cases (15.4%) belonged to the age group 61-80 years. Females were more commonly affected than males with Male:Female ratio of 1:12.

Table 26. Distribution of class IV according to gender (n=13)

Gender	No of Cases	Percentage
Male	1	7.6
Female	12	92.4

The most common clinical presentation observed in these cases were oedema in 9 cases (69.2%) followed by synovitis in 7 cases (53.8%), fever in 6 cases, non-scarring alopecia in 5 cases (38.4%) and oral/nasal ulcers in 4 cases (30.7%). Malar rash was seen in 2 cases and serositis in 3 cases. Hematuria and pain abdomen were observed in 2 cases each.

On evaluation of the blood counts, 7 patients (53.8%) had anemia of moderate degree with hemoglobin levels between 8-10 mg/dl. Severe anemia (Hb <8g/dl) was found in 3 cases (23.1%) and mild degree of anemia in 3 cases i.e. hemoglobin levels between 10-12 g/dl. Leucopenia was seen in 3 cases, one case presented with mild leucocytosis with TLC of $11.5x10^3$/uL. Platelet counts of all these cases were observed to be within normal limits.

Serum creatinine levels of 7 cases (53.9%) were observed to be higher than the normal levels.

Table 27. Distribution of class IV cases according to morphological features (n=13)

	Morphological feature	No of cases	Percentage
Glomerular lesions	Focal involvement	0	0
	Diffuse involvement	13	100
	Global glomerulosclerosis	4	30.7
	Segmental glomerulosclerosis	3	23.0
	Mesangial hypercellularity	13	100
	Endocapillary hypercellularity	10	76.9
	Leukocyte infiltration/karyorrhexis	13	100
	Crescents	11	84.6
	Basement membrane thickening	6	46.1
	Fibrinoid necrosis	5	38.4

	Wire loop deposits	2	15.3
	Hyaline deposits	1	7.6
Tubules	Tubular atrophy	8	61.5
Interstitium	Interstitial inflammation	12	92.3
	Interstitial fibrosis	9	69.2

Morphologically, proliferative lesions involving more than 50% glomeruli were observed in all the cases. Kidney biopsies of all these cases had mesangial hypercellularity and neutrophilic infiltration in the glomeruli, while 10 cases (76.9%) cases showed glomeruli exhibiting endocapillary hypercellularity along with presence of crescents in 11 cases (84.6%) and interstitial inflammation in 12 cases (92.3%). Irregular basement membrane thickening was seen in 6 cases (46.1%), fibrinoid necrosis in 5 cases (38.4%), wireloop deposits in 2 cases (15.3%) and hyaline thrombi in one case (7.6%). Among the chronicity features, interstitial fibrosis was observed in 9 cases (69.2%) with tubular atrophy and glomerular sclerosis in 8 cases (61.5%) and 7 cases (53.7%) respectively. (Table 27)

Three (11.1%) out of these cases had a crescentic pattern of injury. Crescentic glomerulonephritis is defined as more than 50% glomeruli having presence of crescents.

Table 28. Distribution of class IV cases based on IFTA (n=13)

IFTA%	No of cases	Percentage
<5%	3	23.1
6-25%	9	69.3
26-50%	1	7.6
>50%	0	0

Only one case (7.6%) had IFTA of 40-50%, 9 cases (69.3%) had an IFTA of 6-25% while 3 cases (23.1%) of these cases had no significant IFTA.

Modified activity index of more than 12 was seen in 3 cases (23.1%). Rest of the cases diagnosed as Class IV Lupus Nephritis had Modified activity index of less than 12. (Table 27) Modified chronicity index of 6/12 was seen in one case, 5/12 in one case, 4/12 in two cases, 3/12 in three cases, 2/12 in two cases, 1/12 in two cases and 0/12 in two cases.

Table 29. Distribution of class IV cases based on modified activity score (n=13)

Modified activity index	No of cases	Percentage
0-12	10	76.9
12-24	3	23.1

Among the cases diagnosed as Class IV lupus nephritis, 9 cases (69.3%) showed full house positivity on direct immunofluorescence. Among the rest of the cases, one case showed positivity for IgG, C3c and C1q, other case showed positivity for IgG, IgM and C3c, another one showed positivity for IgG and IgA and one case had positive IgG, IgA, C3c and C1q.

CLASS V LUPUS NEPHRITIS (MEMBRANOUS LUPUS NEPHRITIS) (n=3)

Three cases in our study were diagnosed as Class V LN on kidney biopsy where 2 of these belonged to the age group 21-40 years and one belonged to the age group 41-60 years. Male:Female ratio was 1:2.

On examination of the blood counts, 1 case had moderate anemia, 1 case had mild anemia and one case had hemoglobin levels within normal limits. TLC and platelet counts were within normal limits in all the cases. Raised serum creatinine levels were observed in 2 cases.

Table 30. Distribution of class V cases based on morphological features (n=3)

	Morphological feature	No of cases	Percentage
Glomerular lesions	Mesangial hypercellularity	3	100
	Endocapillary hypercellularity	0	0
	Leukocyte infiltration/karyorrhexis	0	0
	Fibrous Crescents	0	0
	Basement membrane thickening	3	100
	Fibrinoid necrosis	0	0
	Wireloop deposits	0	0

	Hyaline deposits	0	0
Tubules	Tubular atrophy	2	66.7
Interstitium	Interstitial inflammation	2	66.7
	Interstitial fibrosis	2	66.7

Morphologically, kidney biopsy of all these patients showed presence of diffuse basement membrane thickening of the glomerular capillary walls. Two cases showed rigid ectatic glomerular capillaries without any endocapillary or extracapillary proliferation. Mild increase in mesangial cellularity was seen in all three cases. Among the chronicity features, tubular atrophy and interstitial fibrosis were seen in 2 cases each. Lymphomononuclear interstitial inflammatory infiltrate was seen in the kidney biopsy of one of these cases while the other two cases had no significant interstitial inflammatory infiltrate. In one case, the tubules showed foamy change with presence of oxalic acid crystals. Blood vessels in 2 cases were histologically unremarkable while in one case there was mild medial hypertrophy of the blood vessels. (Table 30)

Jones' silver stain highlighted argyrophilic spikes in all the cases.

Table 31. Distribution of class V cases based on IFTA (n=3)

IFTA%	No of cases	Percentage
<5%	2	66.7
6-25%	1	33.3
26-50%	0	0
>50%	0	0

One of the cases classified as class V lupus nephritis had 25% IFTA and the rest of the cases had no significant IFTA.

All the cases had a modified activity index of less than 12. Modified chronicity index of 2/12 in one case and 0/12 in two cases.

Among the cases diagnosed as class V lupus nephritis, 2 cases (66.7%) showed full house positivity on direct immunofluorescence while one case showed positivity for IgG, C1q, kappa and lambda.

CLASS VI LUPUS NEPHRITIS (ADVANCED SCLEROSING LUPUS NEPHRITIS) (n=1)

Only a single case in our study was diagnosed as Class VI lupus nephritis. This patient presented with fever, malar rash and synovitis. Blood counts showed moderate anemia with hemoglobin levels of 8.5mg/dl. TLC and platelet counts were within normal limits while serum creatinine levels were raised.

Morphologically, the kidney biopsy of this patient showed 17 glomeruli out of which 16 (94.1%) were totally sclerosed. The only viable glomerulus showed increase in mesangial matrix and cellularity along with lobular accentuation and endocapillary hypercellularity. Neutrophilic infiltration was also identified.

Periglomerular fibrosis with dense lymphocytic infiltrate was seen. Small arteries and arterioles showed marked intimo-medial thickening, sclerosis and luminal narrowing.
IFTA was seen in 55-60% of the sampled cortex which was highlighted on Masson trichrome stain. Modified activity index of 2/24 and modified chronicity index of 9/12 were seen.
On direct immunofluorescence no glomerular deposits were identified.

CLASS III+V (n=3)

Three cases were classified as Class III+V in our study. One of these cases was a male patient of 17 years of age who presented with fever, malar rash, alopecia and synovitis. Blood counts revealed moderate anemia with hemoglobin levels of 9.8g/dl with leucocytosis and platelet counts within normal limits. Serum creatinine levels were within normal limits.
The other case was of a 45 years old female who presented with malar rash, synovitis, oedema and hematuria. Blood counts revealed that the patient had severe anemia with hemoglobin levels of 6.6 g/dl with total leucocyte count and platelet counts within normal limits. Serum creatinine levels were raised.
The third case was a 35 year old female presented with malar rash, oral ulcers, alopecia, synovitis. On evaluation of the blood counts, Hb was 8.6g/dl with TLC and platelet counts within normal limits. Serum creatinine levels were raised.

Morphologically, in all the three cases, the kidney biopsies showed diffuse thickening of the glomerular capillary basement membranes with proliferative lesions in less than 50% glomeruli. In one of the cases, out of the 13 glomeruli, neutrophilic infiltration was seen in 3 glomeruli and endocapillary hypercellularity in 1 glomerulus. In the other case, out of 11 glomeruli, neutrophilic infiltration was seen in 2 glomeruli with endocapillary hypercellularity in 1 glomerulus and presence of short cellular crescent in 1 glomerulus with occasional wireloop deposits. In the third case, 9 out of 15 glomeruli (60%)

showed global sclerosis and were entrapped in interstitial scar. Presence of fibrous crescent was seen in this case.

In one case, the interstitium showed edema with presence of mixed inflammatory infiltrate along with foamy histiocytes while the interstitium in the other case had infiltration by chronic inflammatory infiltrate. The interstitium in the third case was histologically unremarkable. Focal tubular atrophy was seen in one case. Blood vessels were histologically unremarkable in two cases while in the third case small sized arteries showed intimal sclerosis with luminal narrowing.
Argyrophilic spikes were highlighted on Jones silver stains in all the cases.
IFTA was insignificant in 2 of the cases and was in the range of 0-20% in 1 case while in the third case IFTA was 60%.

All three cases had a modified activity index of less than 12. One case had a modified chronicity index of 1/12, another had a modified chronicity index of 0/12 and the third case had a modified chronicity index of 10/12.
Among the 3 cases diagnosed as Class III+V Lupus Nephritis, two showed full house positivity on direct immunofluorescence while the other case showed granular positivity for IgG, C3c along the glomerular capillary loops and mesangium.

DISCUSSION

SLE is a chronic autoimmune disease with variable clinical manifestations. Lupus nephritis is one of the most common and serious manifestations of SLE occurring within the first 5 years of onset, in approximately 40-75% patients. In different parts of the world, the prevalence rate of lupus nephritis differs.[38,39]Even in asymptomatic cases, histological evidence of lupus nephritis on biopsy can be detected.[35] Diagnosis of SLE is made on the basis of clinical and serological pictures. Routine laboratory tests including urinalysis and renal function tests determine the presence of renal involvement in the disease process. Indications of renal biopsy are variable. Some studies recommend kidney biopsy if proteinuria is >500 mg/dL in the presence or absence of clinical symptoms while some if renal function tests such as serum creatinine and estimated glomerular filtration rate are impaired with any level of hematuria and proteinuria in the absence of any other cause.[38] The outcome in patients of lupus nephritis is heterogenous with 10-30% of the patients progressing to ESRD.[31] The diversity and complexity of the clinical manifestations of SLE leads to difficulty in determining the activity status of LN and the associated renal damage, hence it is essential to evaluate the disease activity, the degree of renal damage, and the relationships between renal pathology and laboratory indicators in order to improve the treatment protocols and prognosis. Evaluating the renal biopsy therefore becomes an essential part in the management of the patient as it can change the therapeutic approach to a more aggressive one or protect against drug toxicity in inactive patients.

It is recommended in the literature that renal biopsy should be performed in every patient with evidence of clinical lupus nephritis unless the biopsy is strongly contraindicated. Evaluating renal biopsies in these patients have an additional advantage of reporting other lesions that may be seen in SLE patients apart from typical immune related glomerulonephritis such as acute tubulointerstitial nephritis, vascular diseases (e.g, thrombotic microangiopathy, antiphospholipid

nephropathy, or vasculitis), drug induced podocytopathy and lupus podocytopathy. [36]

The present study was conducted in Dayanand Medical College and Hospital between March 2020 to February 2022 comprising one year of prospective study and one year of retrospective study. A total of 27 cases were studied. The sample size of our study is limited as the study period coincided with the pandemic period of COVID 19.

All renal biopsies with a clinical diagnosis of systemic lupus erythematosus and a clinical indication of lupus nephritis were analyzed with respect to light microscopy and immunofluorescence and were correlated with clinical profile. This study was aimed at analyzing the histomorphological and immunofluorescence spectrum of lupus nephritis and their correlation with clinical profile and laboratory parameters. Further these cases were classified into various categories according to the 2018 modification of ISN/RPS 2003 classification. Activity and chronicity indices were calculated which helped to determine severity of the disease and predict the outcome.

Age and Gender

The mean age of patients included in our study was 38.26±14.5 years. The age ranged from 7 years to 73 years. In a study done by Mahmood SN et al the mean age of presentation was 32.51±16.91 years.[40] The average age of patients in a study done by Venishetty H et al was 32.7 ± 10.7 years while in a study done by Hashmi AA et al the mean age was 28.85±12.24 years.[37,41] Maximum number of patients in our study fall in the age group of 21-40 years which is comparable with studies done by Panda SR et al and Islam SMJ et al where the most common age group was observed to be 21-40 years in both the studies.[42,43]

A female preponderance with a male to female ratio of 1:4.4 was observed in our study. Similar results were seen in a study done by Farah RI which included 79 patients and showed a male to female ratio of 1:6.[35] In another study done by Javeed S et al on 58 renal biopsies, male to female ratio of 1:6.5 was observed.[38] Comparable results were observed in a study done by Yong JL et al with a male to female ratio of 1:4.4.[44] These observations are different from that from studies

in Saudi Arabia, Kuwait and Iran where male: female ratios were 1:8.3, 1:9.8 and 1:10 respectively.[45-47] These variations may be explained by the fact that geographical variation poses a significant risk factor for the incidence of lupus nephritis. Similar conclusion was made on a study done by Pan Q et al where they analyzed the data from 41 studies from different parts of China.[48] The variation in the incidence and prevalence of SLE across different geographical regions could be influenced by both genetic and environmental factors. The genetic makeup of populations can influence susceptibility to SLE, while environmental factors such as exposure to ultraviolet radiation, infections, and toxins can trigger its development.[49] Differences in metabolism of sex hormones and/or gonadotropin releasing hormones may be possible explanations for increased frequency of lupus nephritis in females.[42]

Clinical presentations

Multiple symptoms were seen in the patients included in our study, the most common being synovitis which was present in 16 patients (59.2%) and was observed in all the classes of LN. This was followed by fever and oedema in 12 patients (44.4%) each. Fever was also the presenting symptom in patients of all the classes of LN except for class VI while oedema was observed in the patients diagnosed as classes III, IV, V and III+V LN but not in class II and VI LN. Non scarring alopecia was present in 11 patients and was observed in all the classes except for class VI LN. Malar rash was seen in 10 patients (40.7%) and was observed in all the classes except class II LN. Devadass CW in his study on 46 south indian patients also reported the most common presenting symptoms as arthritis (43.5%), dermatological manifestations (39.1%) and fever (30.4%).[50] In another study conducted on 220 pediatric patients by Qiu S et al the most common initial non-renal manifestations were fever (65.45%), rash (60.46%) and alopecia.[51] Yuan F et al in their study on 156 adult patients concluded that the most common clinical manifestations were arthritis (57.69%), malar rash (50.64%), serositis (36.54%), photosensitivity (15.38%), and oral ulcers (10.90%).[52] These findings were comparable with our study

except photosensitivity which was not seen in any of our cases. Our findings were also similar to a study conducted by Sircar D et al on 86 patients where the common clinical manifestations were arthritis (79%), rash (64%), and fever (66%).[53] None of the patients in our study presented with chronic cutaneous lupus including discoid rash, photosensitization etc. in contrast to study done by Al-Jarallah K et al who in their study observed photosensitivity in 48% and discoid rash in 10% of the patients.[46] This could be explained by the small sample size of our study.

Laboratory and serological parameters

ANA positivity was detected in all of our cases. In a study done by Qiu S et al along with ANA positivity in 99.09% of the cases, 81.36% of the patients showed positivity for anti-dsDNA which was in agreement with the findings of our study where 70.4% of the cases were positive for anti-dsDNA.[51] Another study done on 40 patients by Venishetty H et al showed 85% positivity for anti-dsDNA which was again in concordance with our study.[41] Similar findings were observed in a study done by Javeed S et al on 58 patients which showed ANA positivity in 52 (89.70%) patients and anti-dsDNA positivity in 49 (84.50%) patients.[38] On the contrary, in a study done by Jagdish GA et al on 60 patients anti-dsDNA positivity was seen in a smaller percentage of cases (46.3%).[54] Positivity of anti-dsDNA indicated the background of systemic lupus erythematosus with renal involvement.[38] The most frequent autoantibody found in the present study was anti-dsDNA followed by anti-nucleosome in 14 cases (51.8%), anti-Smith antibodies in 13 cases (48.1%), anti-histone in 12 cases (44.4%), anti SSA in 9 cases (33.3%) and anti SSB in 8 cases (29.6%). Anti scl antibody was negative in all the cases. Our findings are not in concordance with a study done by Talukdar D et al in 2020 on 145 patients where they observed the most frequent autoantibody other than anti-dsDNA to be anti SSA with a frequency of 49.66% followed by anti-Rib-P with a frequency of 30.34%. Anti-nucleosome antibody in their study was seen in only 24.12% of the cases while anti smith antibody was seen in 29.66% of the cases.[55]

Severe anaemia was more frequently observed in patients with class IV LN which is in concordance with studies done by Shobha V et al on 32 patients from Karnataka.[56] Similar results were obtained in the study done by Panda SR et al and Venishetty H et al[41,42]. The correlation between severe anemia and class IV LN can be attributed to an increased risk of developing anemia due to renal failure and active hematuria in patients with class IV LN.

In order to identify the root cause of anemia, a comprehensive hematological examination is required. This examination should include serum iron studies, serum vit B12, serum folate levels, Coombs test, bone marrow study to examine for any suppression caused by medication. Measuring erythropoietin levels is also suggested as part of the evaluation.[57]

In the present study, we investigated 27 cases and found that isolated anemia was the most common hematological abnormality, with 19 cases (70.3%) presenting with this condition. In patients with isolated anemia, 47.3% were diagnosed with Class IV lupus nephritis, followed by 21% with Class III LN and 15.7% with Class V LN. Bicytopenia was present in 6 cases, with 50% of those cases being diagnosed with class IV LN and the remaining cases being diagnosed with class II, III, and V. Only 1 case showed pancytopenia, which was associated with class II LN.

Five cases (18.5%) had thrombocytopenia which is a contraindication for interventional procedures and biopsies are not performed when a patient has moderate to severe thrombocytopenia. This may explain the low number of cases of thrombocytopenia in the present study. Farah RI et al also reported a small number of cases (6%) of thrombocytopenia in their study on 79 Jordanian patients.[35] The most probable cause of thrombocytopenia in systemic lupus erythematosus (SLE) is increased peripheral destruction of platelets and the presence of anti-platelet antibodies. Additionally, antibodies against thrombopoietin, the thrombopoietin receptor c-Mpl, and CD40L have been found in some SLE patients with thrombocytopenia.[58]

Leucocytosis was observed in 14.8% of the cases in the present study. Talukdar D et al also observed leucocytosis in 12.6% of the cases.[55] Leucocytosis can be caused by infections or high doses of steroids. It can also occur during acute flare-ups of systemic lupus erythematosus (SLE).[57]

Among the 27 cases in present study, nephrotic range proteinuria was observed in 48.2% cases while sub-nephrotic range proteinuria was observed in 51.8% of the cases which is in agreement with findings of study done by Qiu S et al where 44.69% of the patients had nephrotic range proteinuria.[51] In a study done by Javeed S et al subnephrotic range proteinuria was the more common presentation seen in 75.9% cases while nephrotic range proteinuria was seen in 24.1% cases.[38] Contrary to our study Jindal B et al found nephrotic range proteinuria in 66% cases.[59] In our study we observed 53.8% cases of class IV LN presenting with subnephrotic range proteinuria while it was the predominant presentation in patients of class IV LN in a study done by Satish S et al.[60] Significant association of class V LN with nephrotic range proteinuria has been reported in previous studies. In the present study out of the 3 cases classified as class V LN, 2 cases were observed to have nephrotic range proteinuria while the third case had subnephrotic range proteinuria.[47,60]

One of the strong adverse clinical prognostic indicators of renal failure is an elevated serum creatinine level at the time of renal biopsy. It is associated with an increased rate of the development of renal failure.[47][60] Raised serum creatinine levels were observed in 13 cases. Studies done by Venishetty H et al, Panda SR et al and Shobha V et al reported highest serum creatinine levels in class IV as compared to other classes while in the present study 53.8% cases of class IV LN had elevated serum creatinine levels.[41,42,56] In the present study both the cases of class II LN have normal serum creatinine levels which is in concordance to the studies done by Venishetty H et al and Devadass CW who reported no increase in serum creatinine in cases classified as class II while an elevated serum creatinine levels was observed in 50% of the cases classified as class II LN in a study done by Gladman DD

et al.[41,50,61] Four out of five cases of class III LN and 6 out of 13 cases of class IV LN presented with normal serum creatinine levels.

In the present study, 3 cases (11.1%) presented with gross hematuria and microscopic hematuria was reported in 23 (85.1%) out of 27 cases. All the cases of class II, VI and III+IV, 12 out of 13 cases of class IV, 4 out of 5 cases of class III and 1 out of 3 cases of class V had microscopic hematuria. These findings are in concordance with the study done by Nezhad ST et al who reported gross hematuria in 16% and microscopic hematuria in 75.2% cases while it is more than 60% cases with hematuria from the study done in Peru. [47,62]

Numerous studies have demonstrated reduced complement levels in cases of active LN. Low serum complement levels (C3, C4) is one of the criteria for clinically diagnosing systemic lupus erythematosus. Hence monitoring the serum levels of complement has become an essential part of management of SLE patients. Among the total 27 cases in our study, C3 levels were observed to be reduced in 21 cases (77.7%) while serum C4 levels were reduced in 16 cases (59.2%). Only one case was observed to have isolated low C4 levels with normal C3 levels and was classified as class V LN. All of the cases of class III LN, 84.6% cases of class IV LN, 66.7% cases of class V and III+V LN and 50% cases of class II LN had reduced serum C3 levels while 50% cases of class II, 80% cases of class III, 61.5% of class IV, 66.7% of class V and 33.3% of class III+V had reduced C4 levels. The single case in class VI LN had normal levels of C3 and C4. These findings are in concordance with the studies done by Chelliah V who in their study on 50 patients reported that low complement levels were significantly associated with proliferative LN. They reported low C3 levels in 68% of the cases and low C4 levels in 74% of the cases.[36] Our findings are also similar to the studies done by Devadass CW et al, Shobha V et al and Gladman DD et al who reported most number of cases with low C3 and C4 in class IV LN suggesting their correlation with proliferating lesions.[50,56,61]

ANCA positivity was seen in 2 cases in our study with both of them being positive for anti MPO antibodies. Turner-Stokes T conducted a

study that found a higher likelihood of patients with positive anti-neutrophil cytoplasmic antibodies (ANCA) to have class IV lupus nephritis (LN) compared to class V LN. The study also found that most of the patients tested positive for anti-myeloperoxidase antibodies rather than anti-proteinase 3 antibodies.[63] In a study conducted by Pradhan VD et al on 59 patients, 37.3% cases were positive for ANCA with 54.5% cases having anti-myeloperoxidase.[64]

Histopathological profile

Almost half of our cases i.e. 13 cases (48.2%) were found to have Diffuse Lupus Nephritis i.e. Class IV LN. The second most common type of lupus nephritis on biopsy was Focal Lupus Nephritis i.e. Class III LN with 5 cases (18.5%) followed by Class V LN i.e. membranous lupus nephritis which included 3 cases (11.1%). No case of class I and class IV+V lupus nephritis were seen in our study. Similar to our findings, none of the cases were reported as class I LN in the studies done by Shobha V et al and Satish S et al.[56,60] The possible explanation for no case in class I LN could be the relatively asymptomatic course of the disease during the initial stages and a comparatively normal laboratory profile including renal function tests, urinalysis etc and hence renal biopsies are not performed at this stage while the reason for no case of class IV+V LN could be the small sample size. The study done by Venishetty H et al also showed similar findings with class IV being the the most common class (53%) followed by class III (22%), V (10%), II (10%) and I (5%) in descending order.[41] These were similar to studies done by Hashmi AA et al, Islam SMJ et al, Devadass CW et al.[37,43,50] Chelliah V et al also reported class IV as the most common class of LN but they did not report any case of class III LN in their study.[36] In the study done by Panda SR et al the most common class of lupus nephritis was observed to be class IV followed by class V and then class III.[42] However, our findings differed from the study done by Javeed S et al who concluded that highest frequency was found of class V LN, 25 (43.10%), followed by class IV, 16 (27.58%).[38]

Overlapping features were seen in 3 cases (7.4%) which included class III+V LN which is different from a study done by Islam SMJ et al where they reported four cases of IV+V LN and two cases of III+V LN in a study on 104 cases.[43] Farah RI et al reported overlapping LN classes in 8.9% of cases, Hashmi AA et al reported combination in 6 cases and Singh U et al reported combination in 3.84% of cases.[35,37,65]

Table 32. Comparison of morphological spectrum of lupus nephritis in various studies

Class	Present study (n=27)	Hashmi AA et al (n=128)[37]	Venishetty H et al (n= 43)[41]	Panda SR et al (n=35)[42]	Islam SMJ et al (n=104)[43]	Devadass CW et al (n=46)[50]
I	0	4	2	0	4	1
II	2	22	4	3	22	9
III	5	14	9	6	25	10
IV	13	60	24	19	38	17
V	3	22	4	7	8	8
VI	1	0	0	0	1	1
III+V	3	0	0	0	2	0
IV+V	0	6	0	0	4	0

In the present study there was no case of class I LN. Renal biopsy in both the cases of class II LN showed mesangial hypercellularity with no evidence of active or chronic lesions. Suchitha Satish et al in their study reported 4 cases of class II LN all of which showed mesangial hypercellularity. They also reported glomerular sclerosis, tubular atrophy and interstitial inflammation in 25% of cases of class II LN while it was not reported in any cases of class II LN in the present study. [60]

Satish S et al reported 13 cases of class III LN with 100% of their cases showing mesangial hypercellularity and endocapillary

proliferation, 76.9% cases with leucocytic infiltrate, 15.4% cases with wire loop deposits and cellular crescents in 7.7% cases. Interstitial inflammation was seen in 38.5%, glomerular sclerosis in 42.9%, fibrous crescents in 15.4% and tubular atrophy and interstitial fibrosis each in 15.4% cases. In the present study 5 cases of class III LN were reported with all of them having mesangial hypercellularity and leucocytic infiltration while endocapillary proliferation was seen in 80% of the cases and cellular crescents in 40% of the cases. Fibrinoid necrosis was seen in 1 case of class III LN in the present which was not reported in any case of class III LN in the study done by Satish S et al. Interstitial inflammation was seen in 60% and tubular atrophy and interstitial fibrosis in 20% of the cases. Basement membrane thickening was seen in 40% of the cases of class III LN which was not reported in any case of class III LN by Satish S et al. There was no glomerular sclerosis or fibrous crescents in any case of class III LN in the present study.[60]

Satish S et al reported 31 cases of class IV LN with 100% of the cases having mesangial hypercellularity and endocapillary proliferation. 93.5% cases having leucocytic infiltration, 64.5% cases with wireloop deposits, 6.5% cases with fibrinoid necrosis and 41.9% cases with cellular crescents. Interstitial inflammation was seen in 80.6% cases, glomerular sclerosis in 35.5% cases, fibrous crescents in 9.7%, tubular atrophy and interstitial fibrosis in 35.5% cases. The present study had 13 cases of class IV LN all of which displayed mesangial hypercellularity and leucocytic infiltration, endocapillary hypercellularity was seen in 76.9% cases, wireloop deposts in 15.3%, fibrinoid necrosis in 38.4% and cellular crescents in 84.6% cases. Interstitial inflammation was seen in 92.3% cases, fibrous crescents in 15.3%, glomerular sclerosis in 30.7% cases and interstitial fibrosis and tubular atrophy in 61.5% cases.[60]

Satish S et al reported 7 cases of class V LN all of which showed mesangial hypercellularity and basement membrane thickening in more than 50% glomeruli similar to the present study. However, in the study done by Satish S et al 42.9% cases of class V LN had endocapillary

proliferation and 28.6% cases showed wireloop deposits which were not seen in any case of class V LN In the present study. Satish S et al reported glomerular sclerosis in 57.1%, fibrous crescent in 14.3% cases which were not seen in any case of class V LN in the present study. Interstitial inflammation and fibrosis and tubular atrophy were seen in 66.7% of the cases in the present study which were not reported in any case of class V LN by Satish S et al.[60]

The only case reported as class VI LN by Satish S et al showed diffuse glomerular global sclerosis with interstitial fibrosis and tubular atrophy which is similar to the findings seen in the only case reported as class VI LN in the present study where more than 90% of the glomeruli were sclerosed. In addition the renal biopsy of this case also showed mesangial hypercellularity, endocapillary proliferation and leucocytic infiltrate in an occasional glomerulus.[60]

Active lesions in LN include endocapillary hypercellularity, fibrinoid necrosis, karyorrhexis, extracapillary proliferation, wire-loop deposits while chronic lesions include glomerular sclerosis, interstitial fibrosis, tubular atrophy and fibrous crescents. The active lesions are reversible and respond to therapy while the chronic lesions are irreversible.[60] In the present study the highest activity index was seen in patients classified as class IV LN which is similar to study done by Shobha V et al and Hurtado A et al but these studies also reported highest chronicity in class IV while in our study the highest chronicity index was observed in a patient of class III+V LN.[56,62] Mean activity index in present study was highest in class IV LN while mean chronicity index was highest in class VI followed by class V LN. There was only one case of class VI LN which could be the reason for the highest mean chronicity index. In contrast to our study Venishetty H et al reported mean chronicity index in class III LN but similar to our study they reported highest mean activity index in class IV LN.[41]

Table 33. Comparison of mean activity indices of various classes of lupus nephritis among various studies

Class	Present study (n=27)	Satish S et al (n=56)[60]	Islam SMJ et al (n=104)[43]	Hurtado A et al (n=33)[62]
I	0	0	-	0
II	0	0	-	0.3±0.5
III	5.00±2.55	3.00±1.080	4.59±2.06	-
IV	8.69±3.66	8.13±3.442	8.71±3.41	4.3±3.4
V	2.00±2.65	2.29±2.984	-	0.5
VI	2.00	0	-	0.0
III+V	2.67±1.15	-	-	-
IV+V	0	-	-	-

The term crescentic glomerulonephritis is used when more than 50% of the sampled glomeruli have crescents. In the present study crescent formation was found in 2 out of 5 (40%) cases of class III LN and 11 out of 13 cases (84.6%) of class IV LN. But crescentic lupus nephritis was seen in 3 out of 13 (23%) Class IV LN cases. Similar findings were observed by Singh U et a in their study where they reported 35.71% of Class IV LN as crescentic LN.[65] Yu F et al in their study in China reported crescentic LN In 10% of class IV LN cases.[66]

On evaluating DIF findings, of the 27 cases studied, one could not be evaluated due to inadequate biopsy. Of the remaining 26 cases, one case which was categorized as advanced sclerosing lupus nephritis i.e. Class VI had no glomerular deposits of immunoglobulins, 18 cases (66.6%) showed full house positivity on direct immunofluorescence that is these cases showed glomerular deposits of IgG, IgA, IgM, C1q and C3c which is in concordance with a study done by Nossent H et al

where 46 out of 69 biopsies (67%) showed full house positivity with C3 being the most frequently involved immune deposit in contrast to our study.[67] Full house positivity was seen in 56.5% of the cases in a study done by Devadass CW et al with IgG being the most commonly involved immunoglobulin which is comparable with our study where IgG was seen in 100% of the cases.[50] Our findings are also similar to a study done by Satish S et al where 100% of the biopsies were positive for IgG.[60] Our findings are in contrast to a study done by Javeed S et al who found C1q (96.55%) to be the most common immune deposit followed by IgG (94.83%).[38] Islam SMJ et al in their study on 104 cases also reported full house positivity in the majority of their cases (86%).[43] Twenty four (60%) biopsies in a study done by Venishetty H et al on forty biopsies showed full house positivity on direct immunofluorescence with C1q and IgG being the most commonly involved immune deposit.[41]

Limitations of the study

The limitations of the study mentioned include a small sample size and a lack of long-term follow-up to observe the progression of the disease or potential remission.

Additionally, the study being conducted at a single center may limit the generalizability of the results to the entire population. The results may not accurately reflect the true prevalence of different classes of LN in the country. Furthermore, the absence of electron microscopy at the institute prevents more in-depth analysis of the condition.

It is recommended to implement rigorous protocols for evaluation, diagnosis, and follow-up as LN has high morbidity and mortality rates, leading to end-stage kidney disease. Despite the latest advances in the field, the proper diagnosis and monitoring of patients with LN is crucial to improving outcomes.

SUMMARY AND CONCLUSION

The present study was conducted on 27 renal biopsies that were received with a clinical suspicion of lupus nephritis based upon clinical signs and symptoms and ANA positivity on indirect immunofluorescence, over a period of two years from 1st March 2020 to 28th February 2022 in the department of pathology, Dayanand medical college and hospital, Ludhiana.

Salient observations and interpretations of results of the present study are as under:

- The cases analyzed were in the age range of 7 to 73 years with a mean age of 38.2 years. Highest number of cases belonged to the age of 21-40 years (59.3%).
- Females were more commonly affected with male to female ratio of 1:4.4.
- The commonest clinical presentation was synovitis (59.2%) followed by fever and oedema (44.4%), non-scarring alopecia (40.7%) and malar rash (37%).
- Diffuse lupus nephritis was the most common class seen in 48.2% cases followed by class III LN (18.5%). Overlapping was seen in 11.1% cases i.e. class III+V. The least common class was class I LN.
- Isolated anemia was the most common hematological parameter, affecting 70.3% cases. Bicytopenia was seen in 22.2% cases and pancytopenia was seen in 3.7% cases. One case had normal hematological parameters.
- Severe anemia was more commonly seen in patients classified as class IV lupus nephritis. Of the 5 cases with leucopenia, 3 belonged to class IV lupus nephritis.
- Sub-nephrotic range proteinuria was observed in 51.8% cases while nephrotic range proteinuria was observed in 48.2% cases.
- In our study, we found that 48.1% had elevated serum creatinine levels 18.6% cases having levels higher than 3 mg/dL.
- Microscopic hematuria was present in 23 of the 27 cases (85.1%).

- All the cases were positive for ANA by indirect immunofluorescence and 48.1% tested positive for anti-Smith antibodies.
- Of the total 27 cases, 21 cases (77.7%) had reduced C3 levels and 16 cases (59.2%) had reduced serum C4 levels.
- 66.6% of the cases showed full house positivity i.e. positivity for all the five markers (IgG, IgA, IgM, C1q and C3c) on direct immunofluorescence.
- The highest mean activity index was seen in class IV and class III as 8.6 and 5 respectively while the highest mean chronicity index was seen in class VI which was 9 followed by class III+V and then class IV.
- Active lesions were more commonly seen in class III, IV and III+V lupus nephritis while chronic lesions were more commonly observed in class IV and V lupus nephritis

The renal biopsy represents a gold standard in management of LN. Studies performed so far show an overall greater reproducibility of the ISN/RPS classification than previous classifications. However, precise assessment and identification of key tubulointerstitial and vascular lesions with prognostic importance are lacking. The varying patterns of glomerular injury, whether segmental, dominantly necrotizing, or global proliferative, and type of sclerosis also remain incompletely understood in terms of pathogenesis and prognostic effect. Thus, additional multicentric studies are needed to evaluate the prognostic relevance of the 2003 ISN/RPS classification and add new prognostic biopsy parameters.

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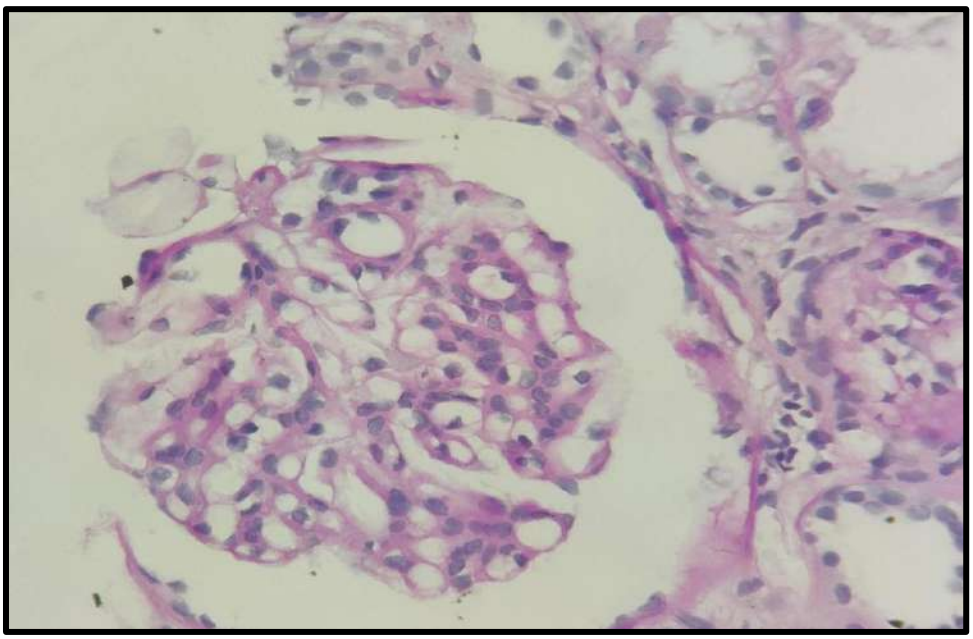
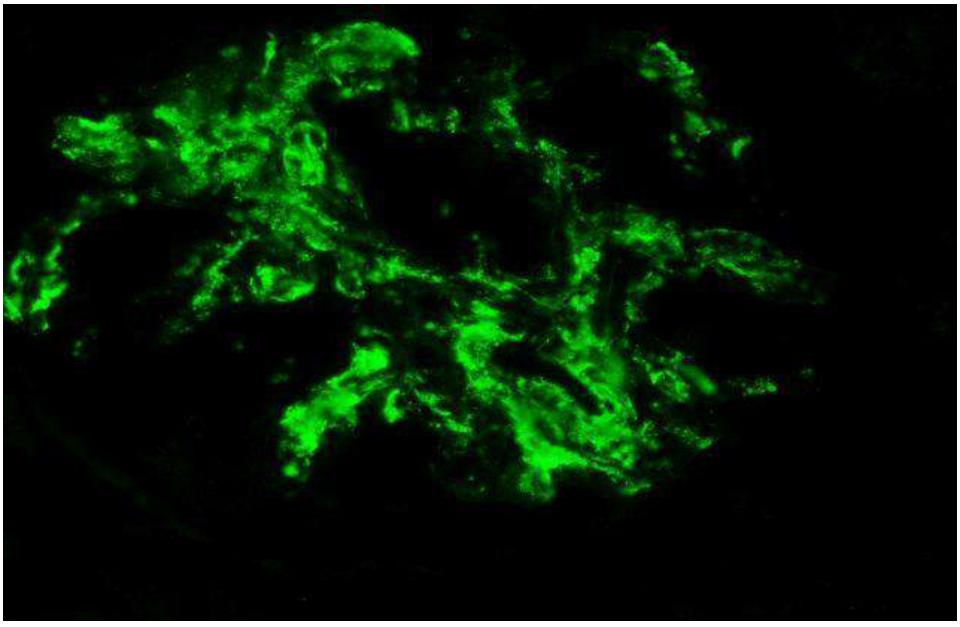
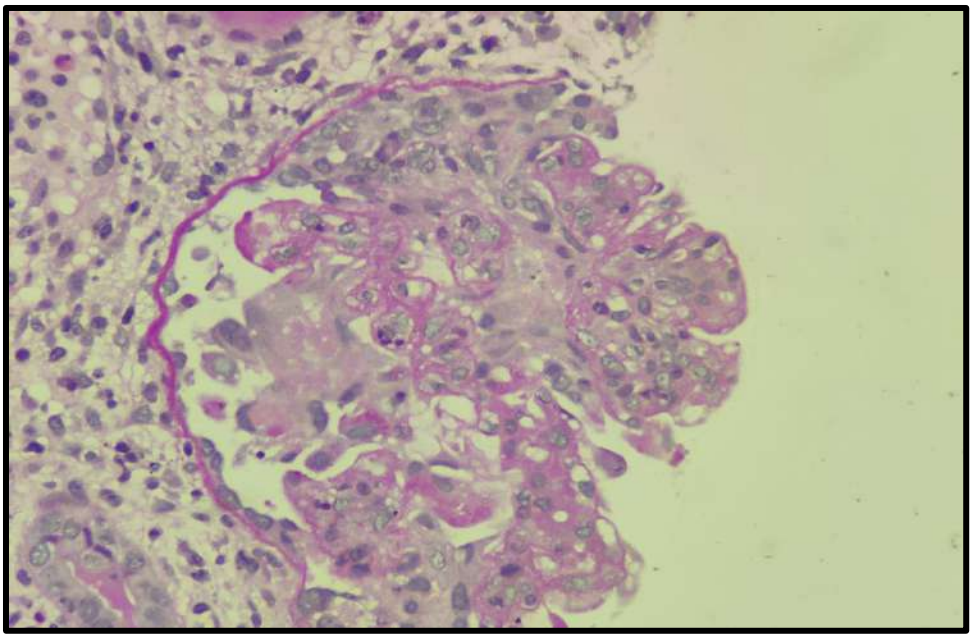


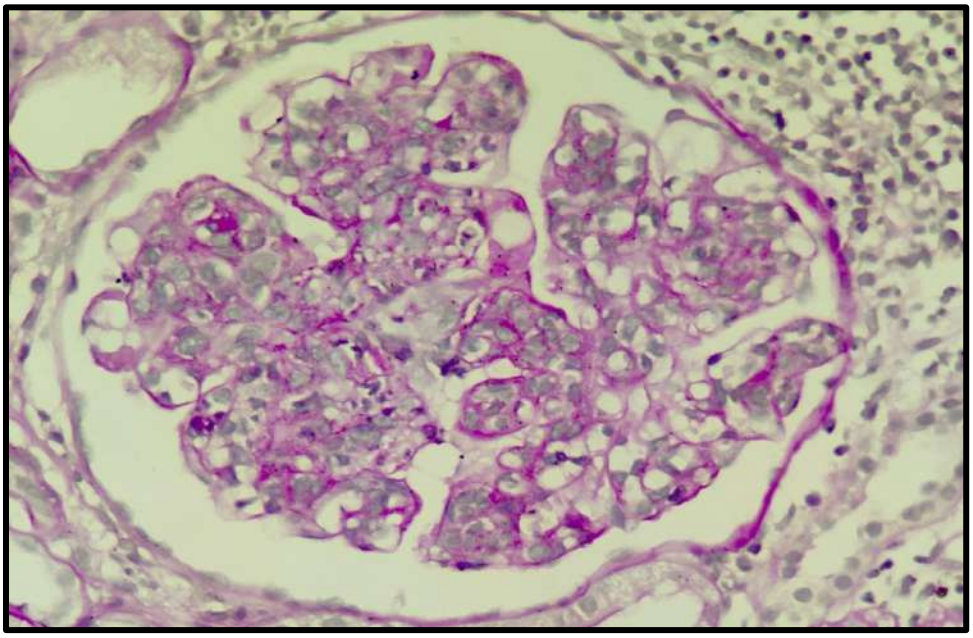
Picture 1-Photomicrograph showing a case of class II LN having mild segmental mesangial hypercellularity (PAS,400X)



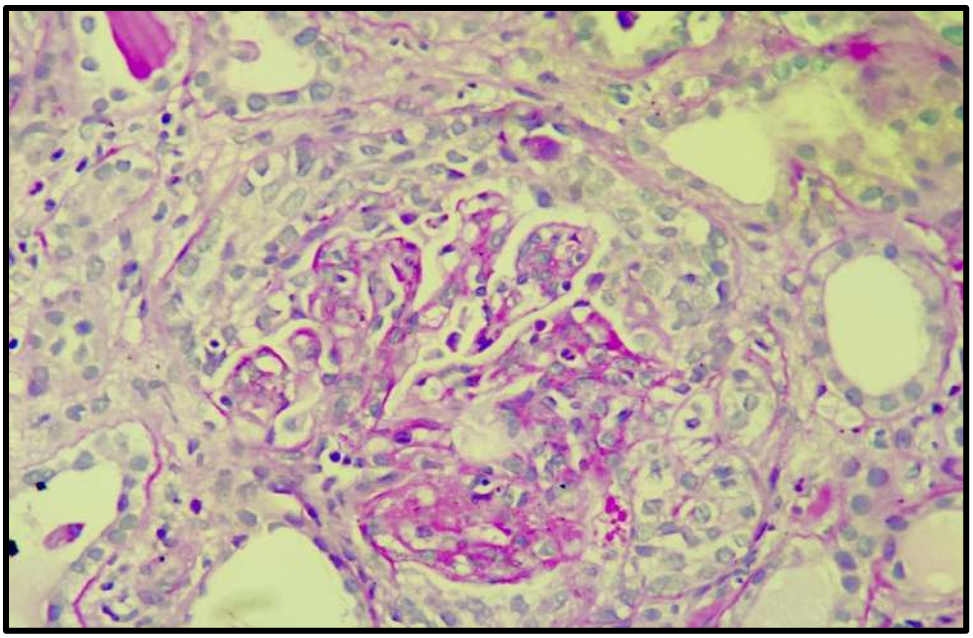
Picture 2-Immunofluorescence of a case of class II LN shows immunoglobulin deposits in the mesangium (anti-IgG immunofluorescence,400X)



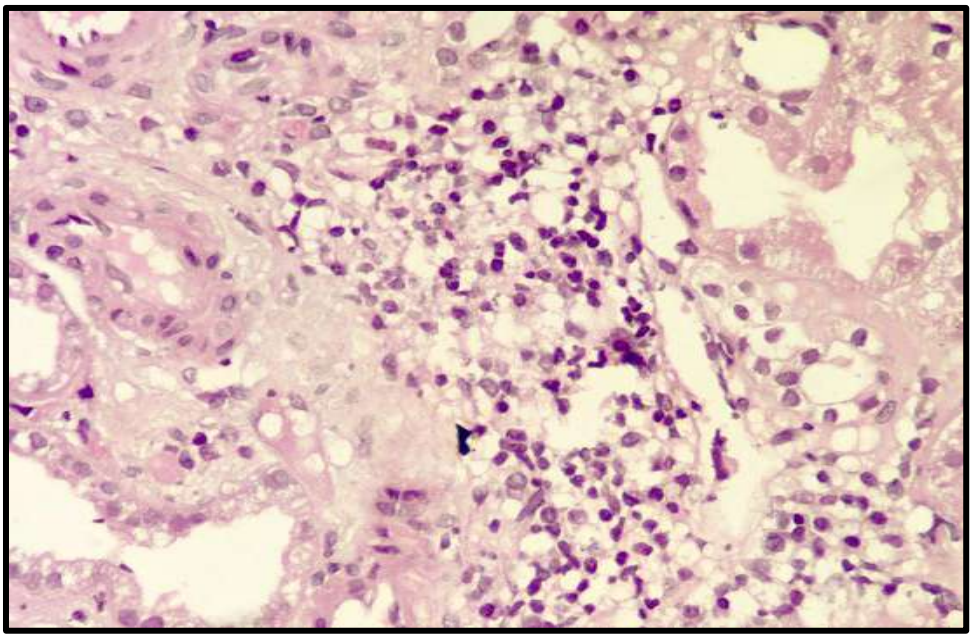
Picture 3-Photomicrograph showing a case of class III LN having mesangial hypercellularity, endocapillary proliferation and a short cellular crescent (PAS, 400X)



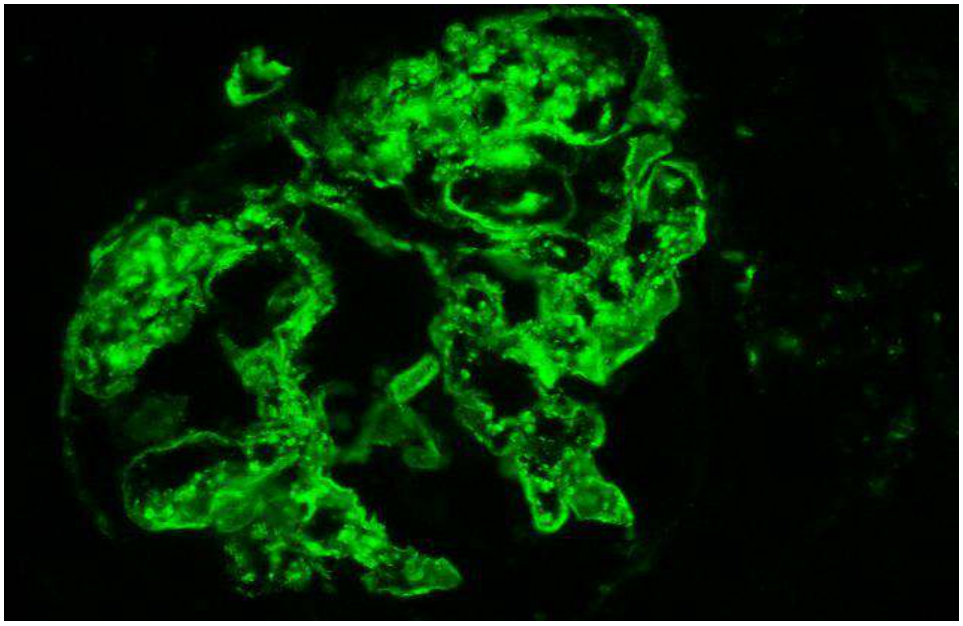
Picture 4-Photomicrograph showing a case of class III LN having lobular accentuation in the glomerulus with mesangial hypercellularity, leucocytic infiltration, endocapillary proliferation and karyorrhectic debris (PAS, 400X)



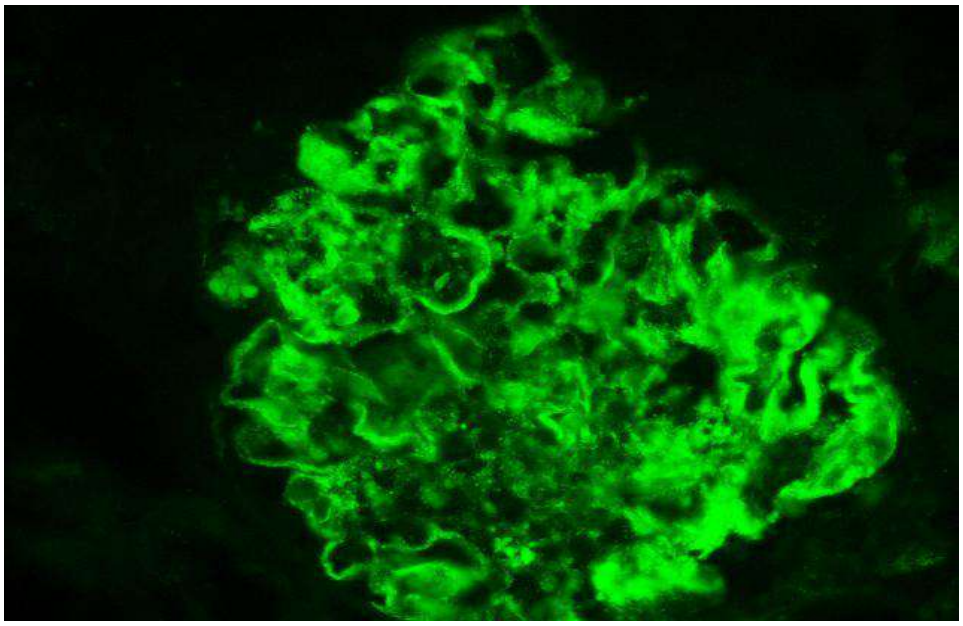
Picture 5-Photomicrograph showing a case of class III LN having a cellular crescent with leucocytic infiltration in the glomerular tuft (PAS, 400X)



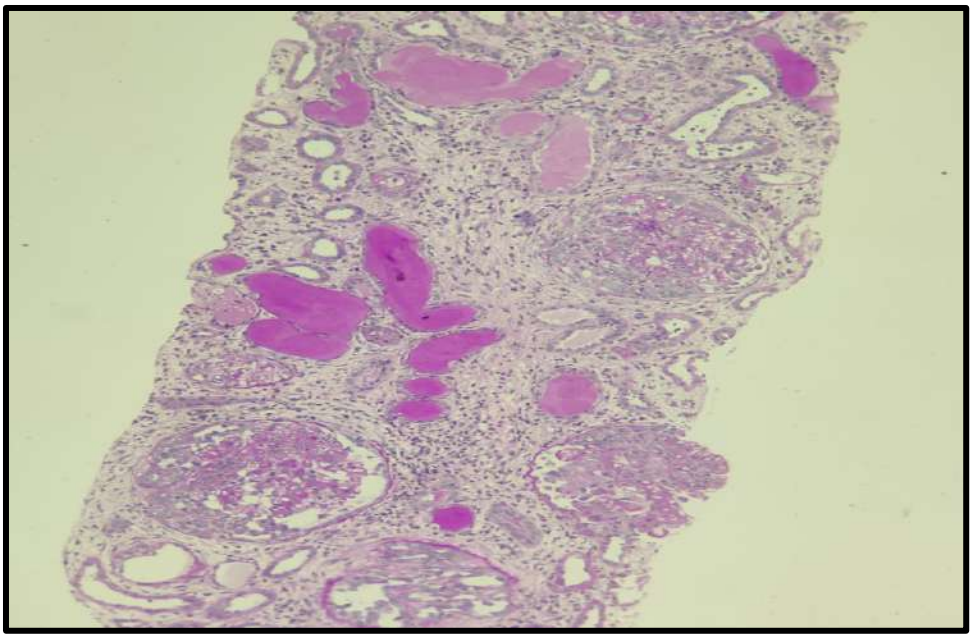
Picture 6-Photomicrograph showing a case of class III LN having interstitial infiltration by chronic inflammatory cells (HE,400X)



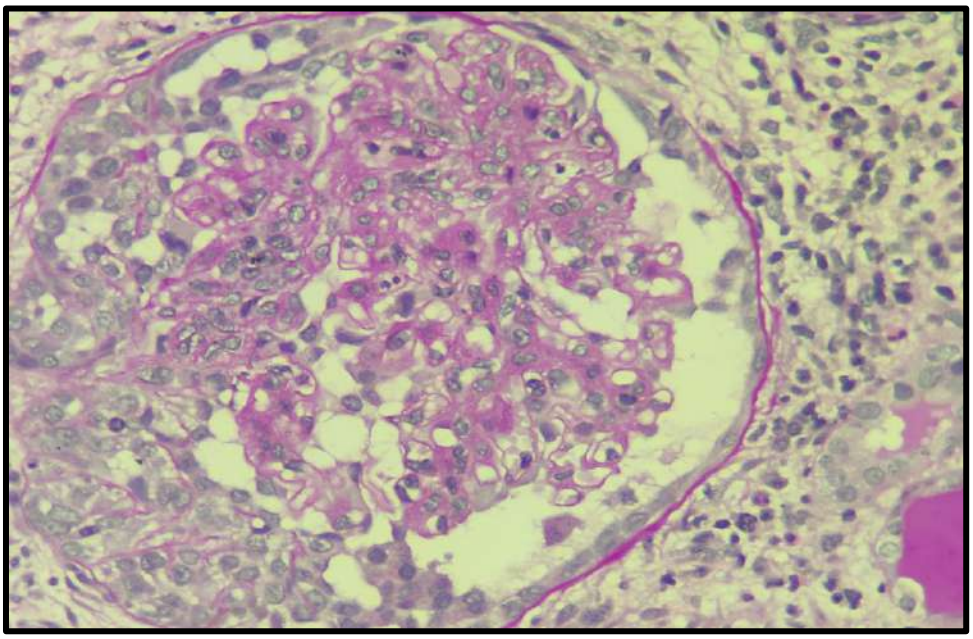
Picture 7-Immunofluorescence of a case of class III LN shows immunoglobulin deposits in the mesangium and along the glomerular capillary loops (anti-IgG immunofluorescence, 400X)



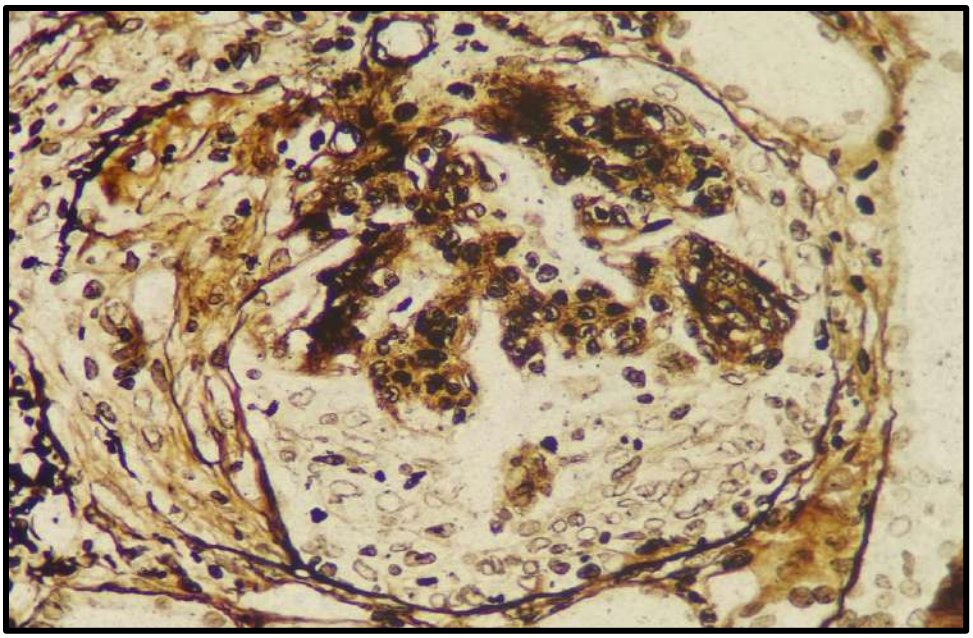
Picture 8-Immunofluorescence of a case of class III LN shows immunoglobulin deposits in the mesangium and along the glomerular capillary loops (anti-C3c immunofluorescence, 400X)



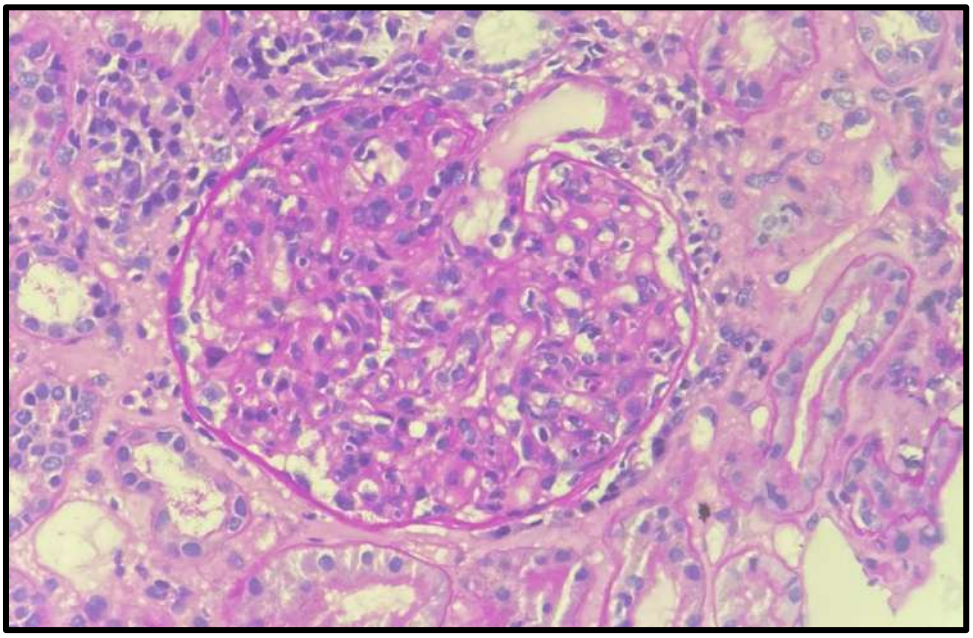
Picture 9-Photomicrograph showing a case of class IV LN with diffuse involvement of the glomeruli having proliferative lesions and hyaline casts in tubules (PAS,100X)



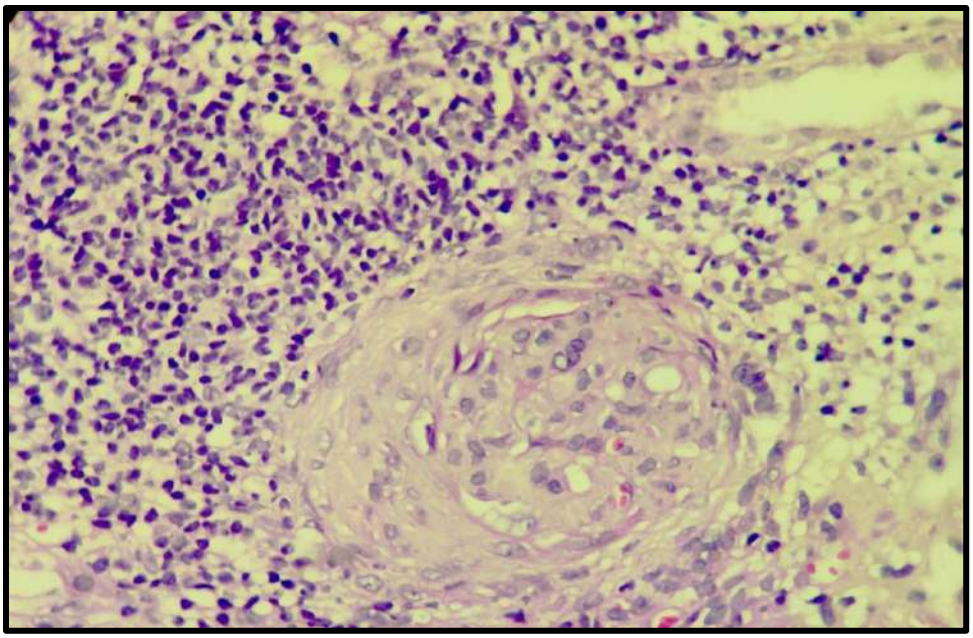
Picture 10-Photomicrograph showing a case of class IV LN having cellular crescent with mesangial hypercellularity and endocapillary proliferation (PAS,400X)



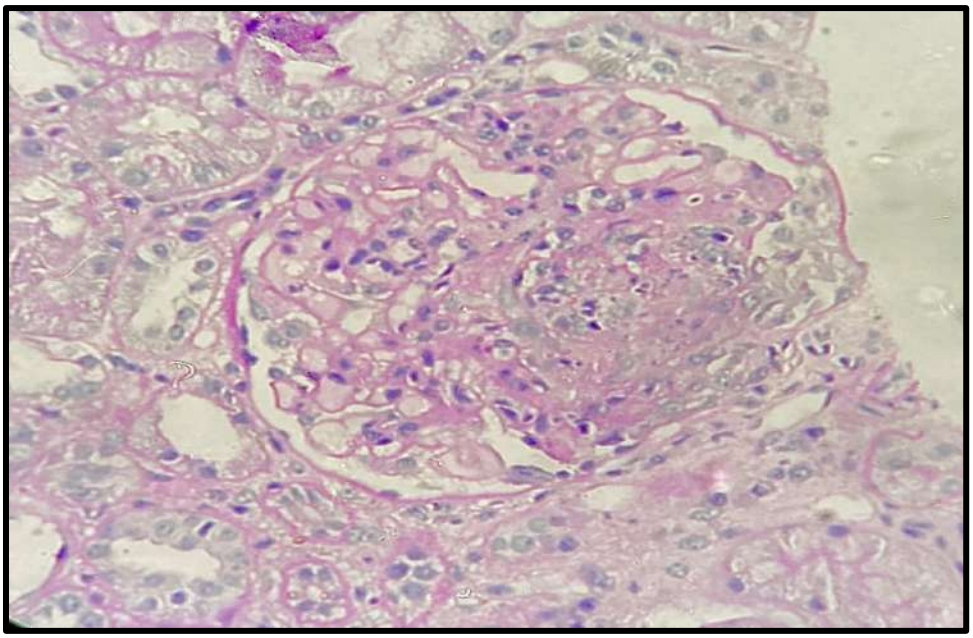
Picture 11-Photomicrograph showing a case of class IV LN having cellular crescent with underlying compressed glomerular tuft (Jones Silver, 400X)



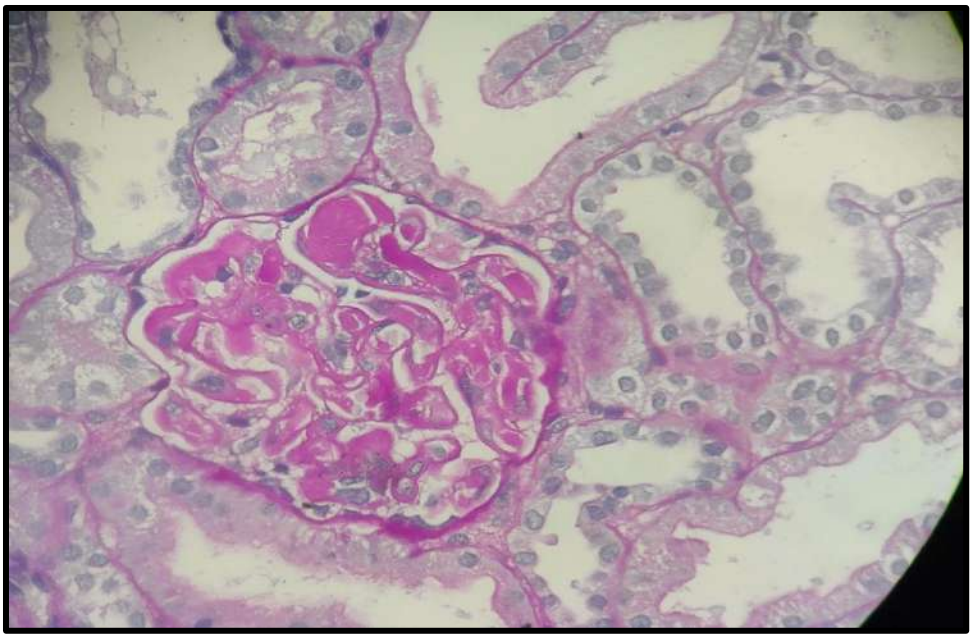
Picture 12-Photomicrograph showing a case of class IV LN with endocapillary proliferation and mesangial hypercellularity (PAS,400X)



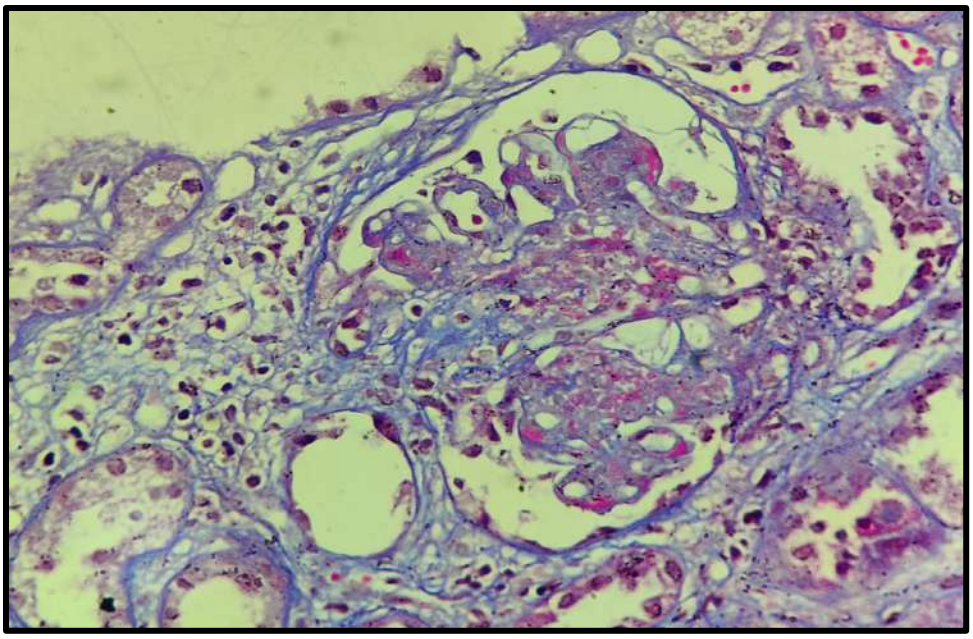
Picture 13- Photomicrograph showing a case of class IV LN having fibrocellular crescent with lymphoid aggregate in the interstitium (PAS,400X)



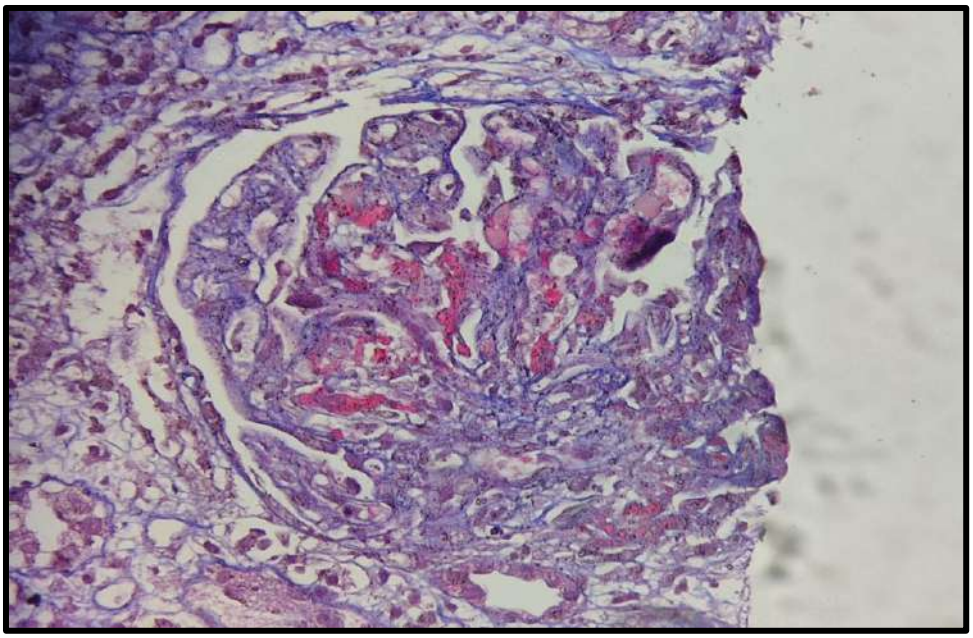
Picture 14-Photomicrograph showing a case of class IV LN having endocapillary proliferation, leucocytic infiltration and karyorrhectic debris (PAS,400X)



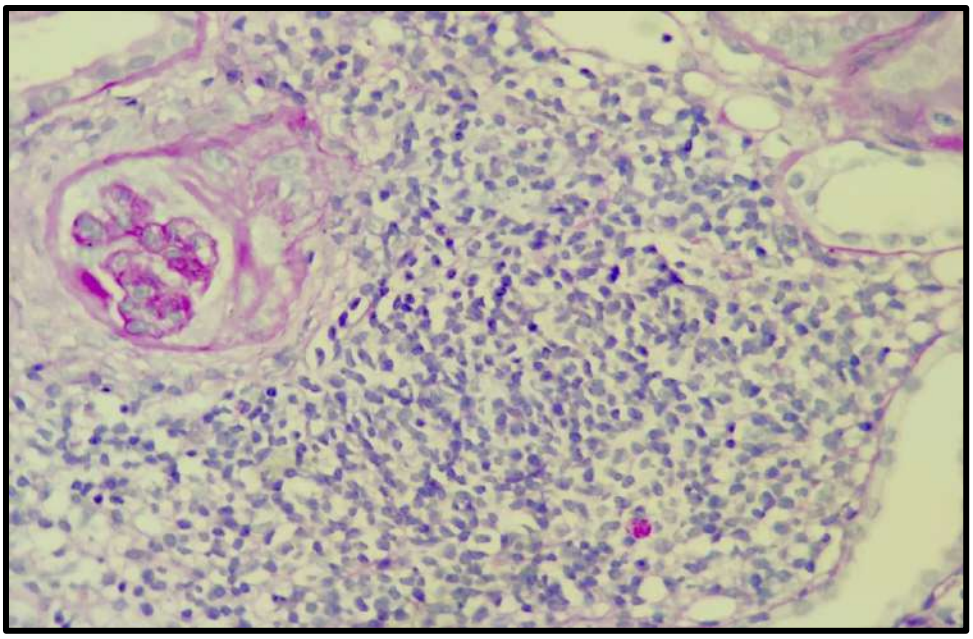
Picture 15- Photomicrograph showing a case of class IV LN having wireloop deposits and hyaline thrombi (PAS,400X)



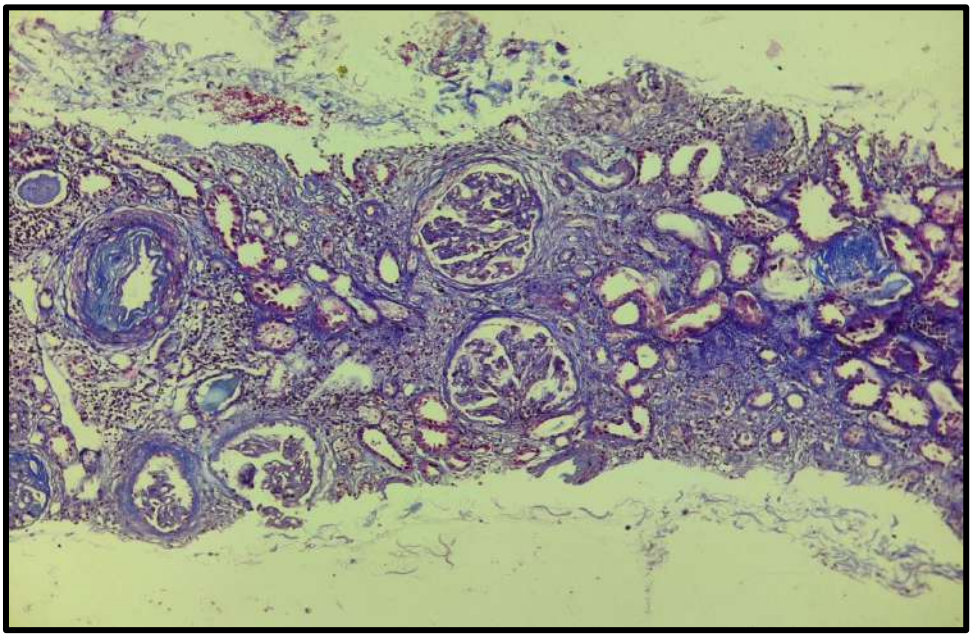
Picture 16-Photomicrograph showing a case of class IV LN having wireloop deposits (MT,400X)



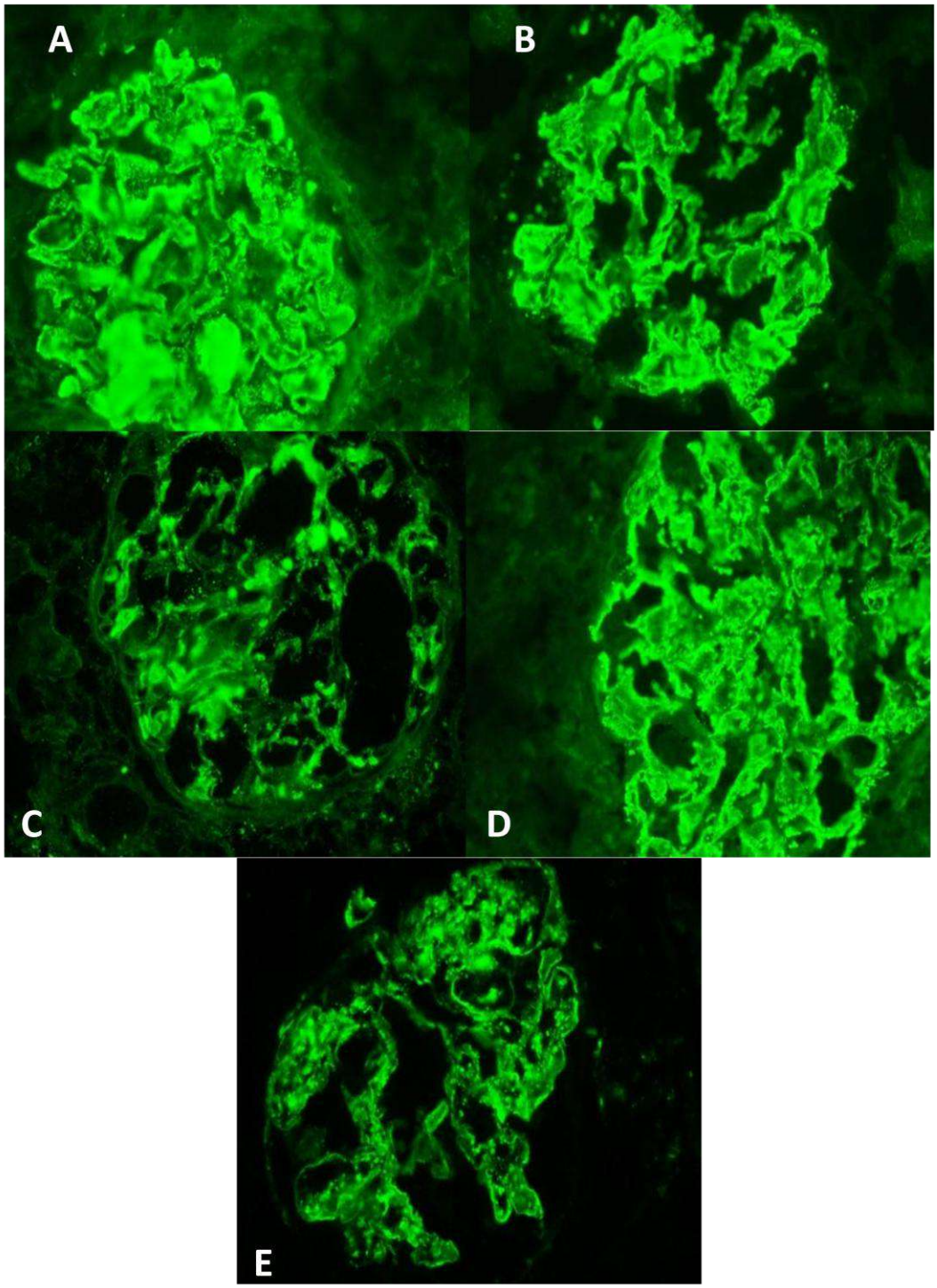
Picture 17- Photomicrograph showing a case of class IV LN having fibrinoid necrosis of the glomeruli (MT,400X)



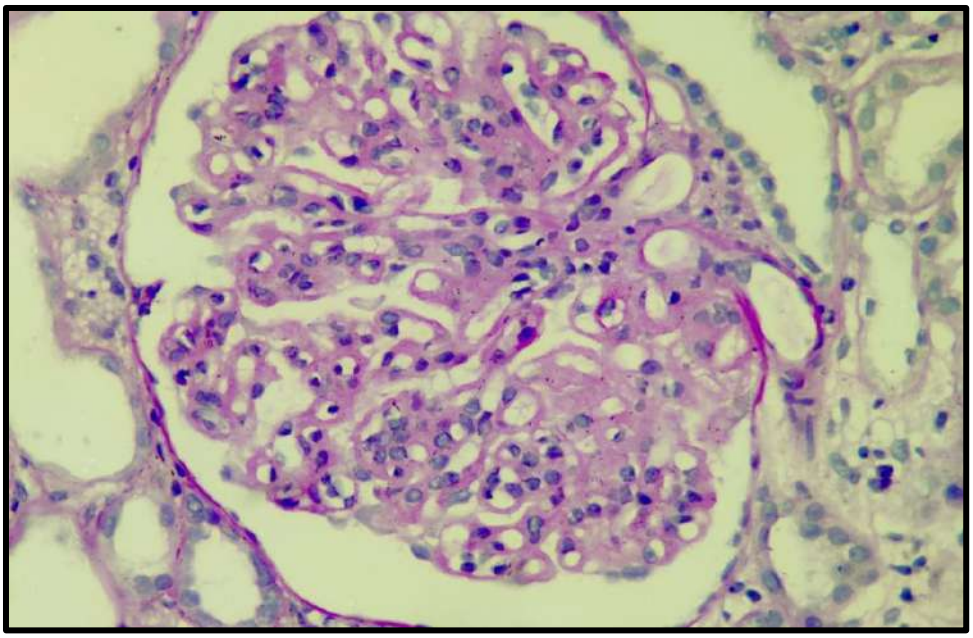
Picture 18-Photomicrograph showing a case of class IV LN having lymphoid aggregate in the interstitium (PAS,400X)



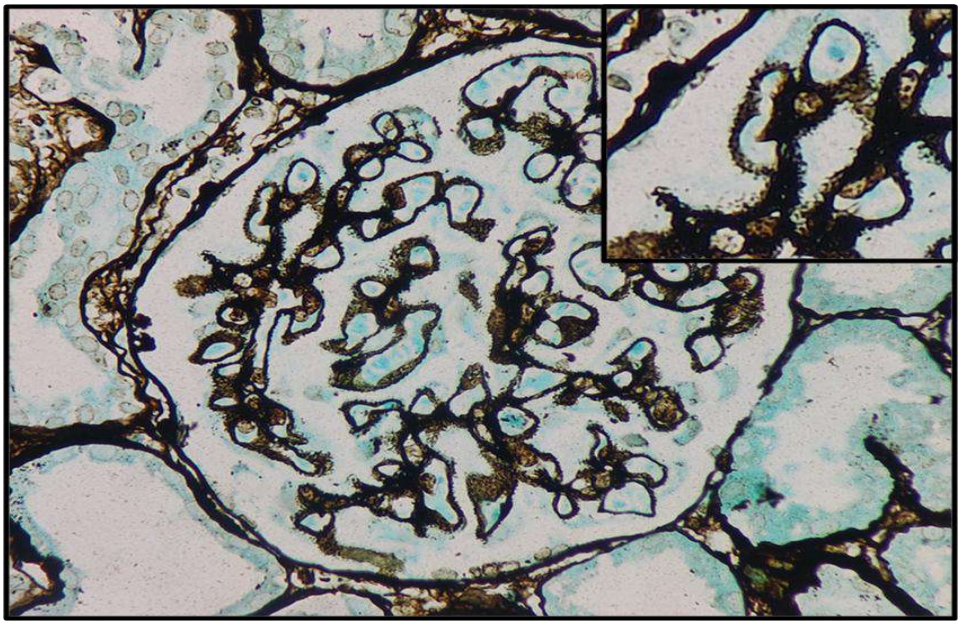
Picture 19-Photomicrograph showing a case of class IV LN having 40-50% IFTA (MT,100X)



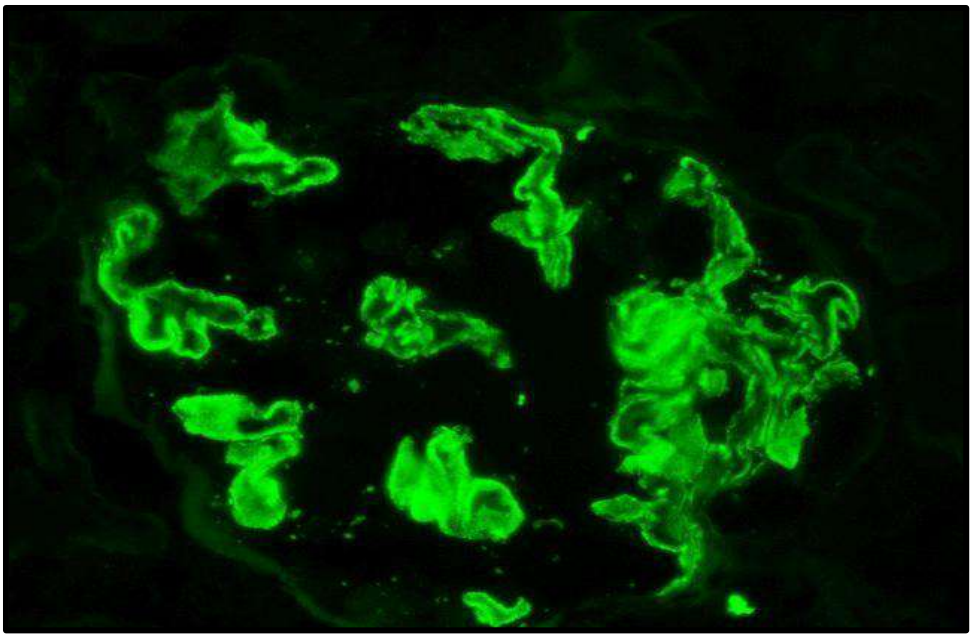
Picture 20- Immunofluorescence of a case of class IV LN shows Full house positivity with immunoglobulin deposits in the mesangium and along the glomerular capillary loops (A-anti IgG, B-anti IgM, C-anti IgA, D-anti C1q, E-anti C3c immunofluorescence, 400X)



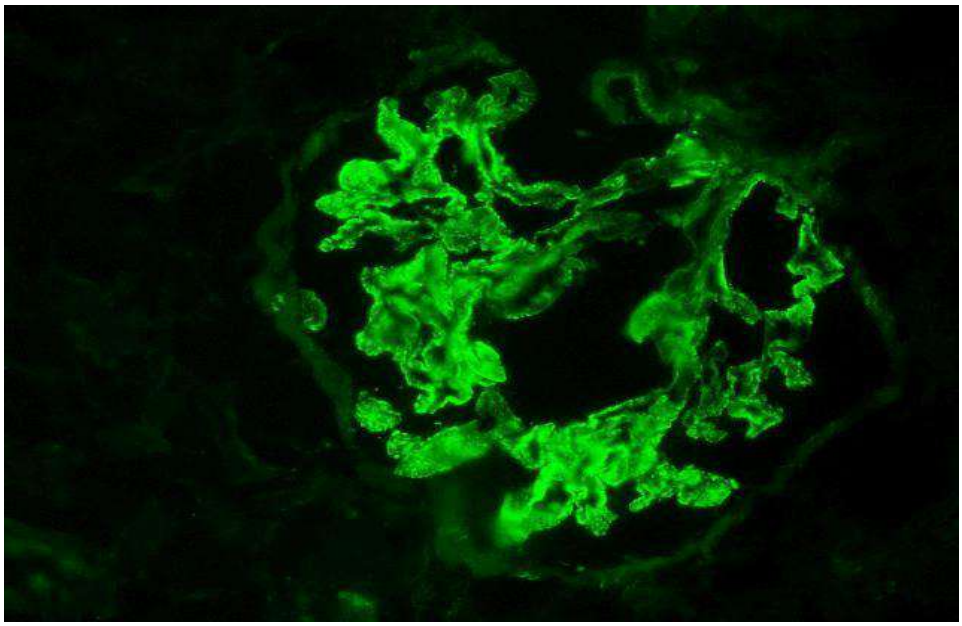
Picture 21-Photomicrograph showing a case of class V LN having basement membrane thickening and mesangial hypercellularity (PAS,400X)



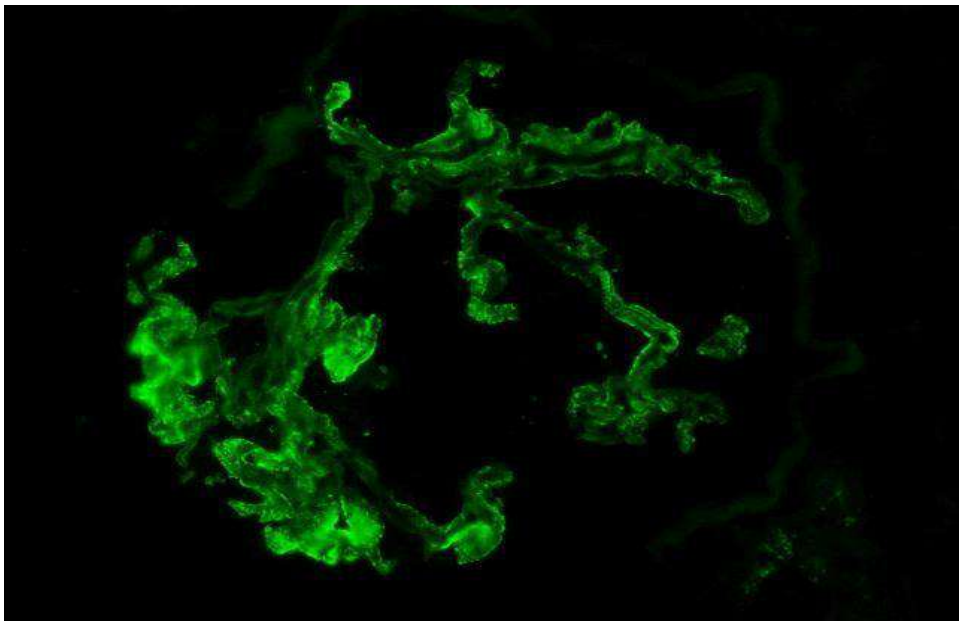
Picture 22-Photomicrograph showing a case of class V LN having argyrophilic spikes (Jones Silver,400X)



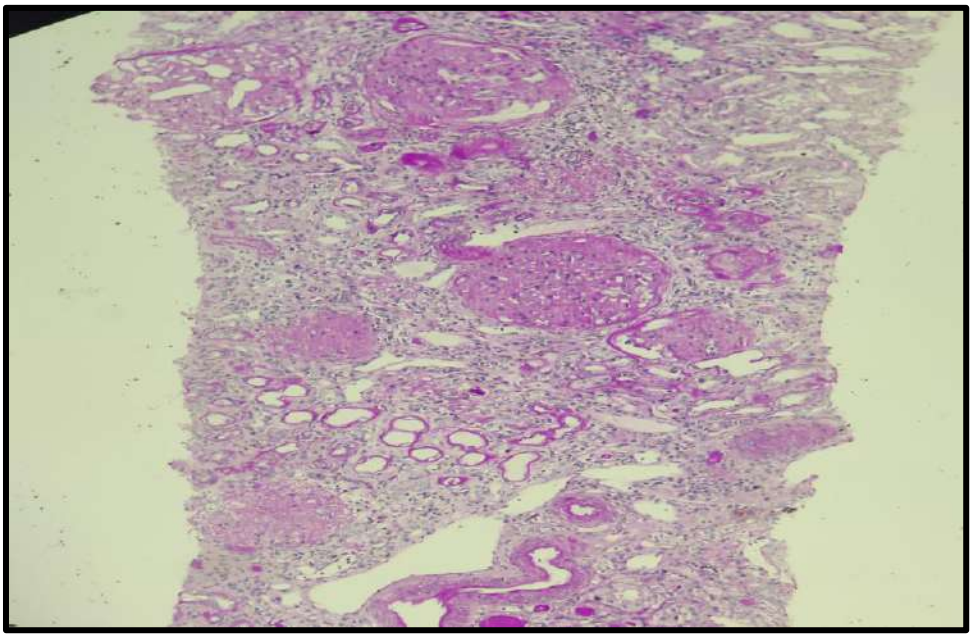
Picture 23-Immunofluorescence of a case of class V LN shows immunoglobulin deposits along the glomerular capillary loops (anti-IgG immunofluorescence, 400X)



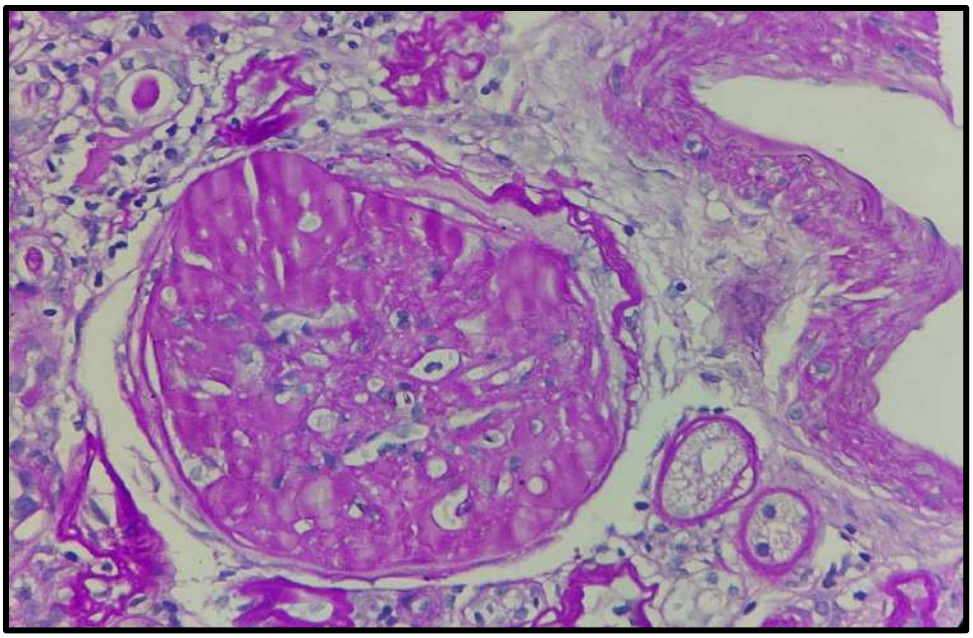
Picture 24-Immunofluorescence of a case of class V LN shows immunoglobulin deposits along the glomerular capillary loops (anti-C1q immunofluorescence, 400X)



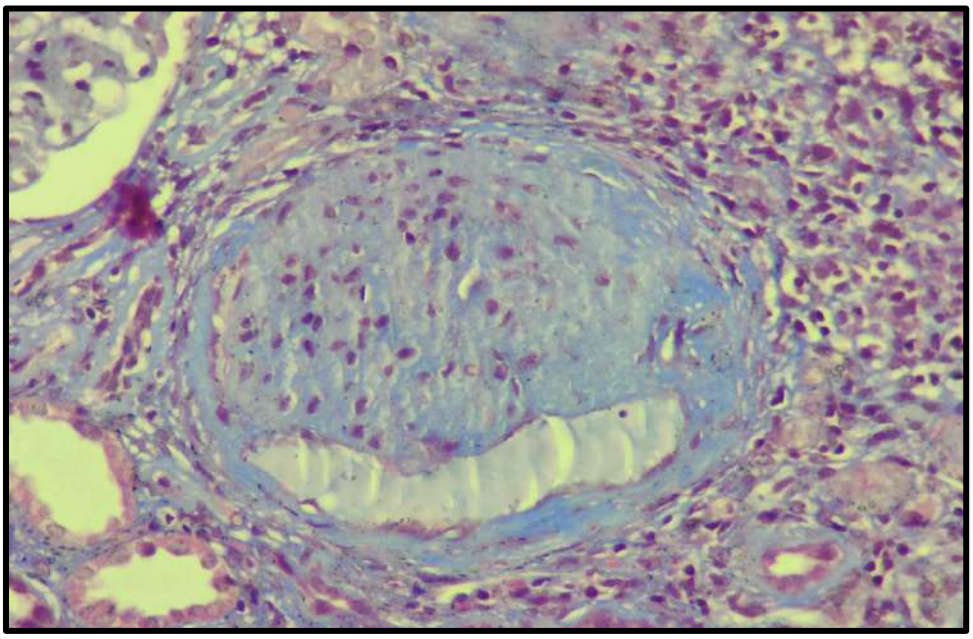
Picture 25- Immunofluorescence of a case of class V LN shows immunoglobulin deposits along the glomerular capillary loops (anti-C3c immunofluorescence, 400X)



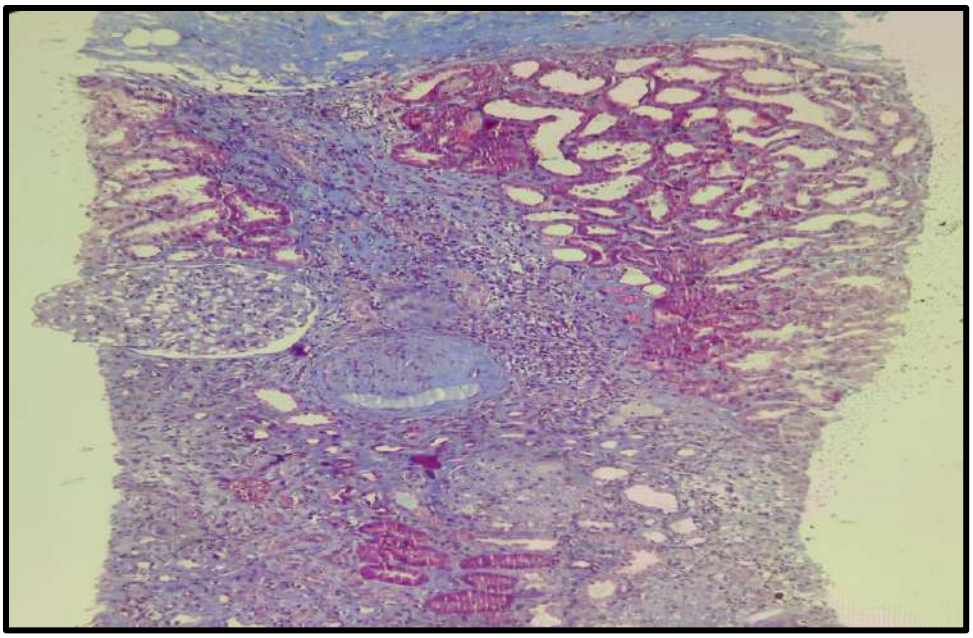
Picture 26- Photomicrograph showing a case of class VI LN having 5 sclerosed glomeruli and one viable glomerulus with tubular atrophy (PAS, 100X)



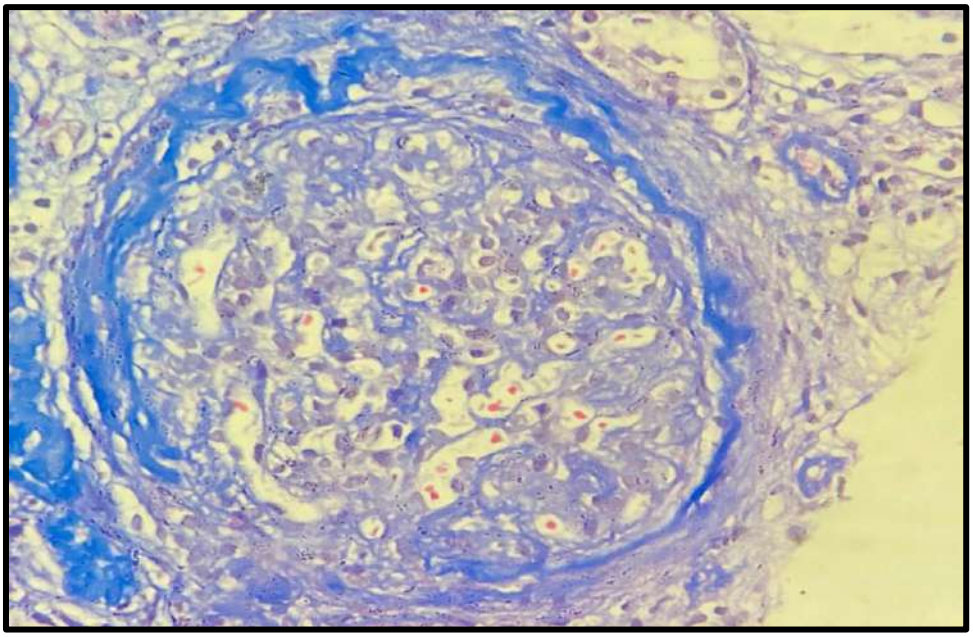
Picture 27- Photomicrograph showing a case of class VI LN having sclerosed glomerulus (PAS,400X)



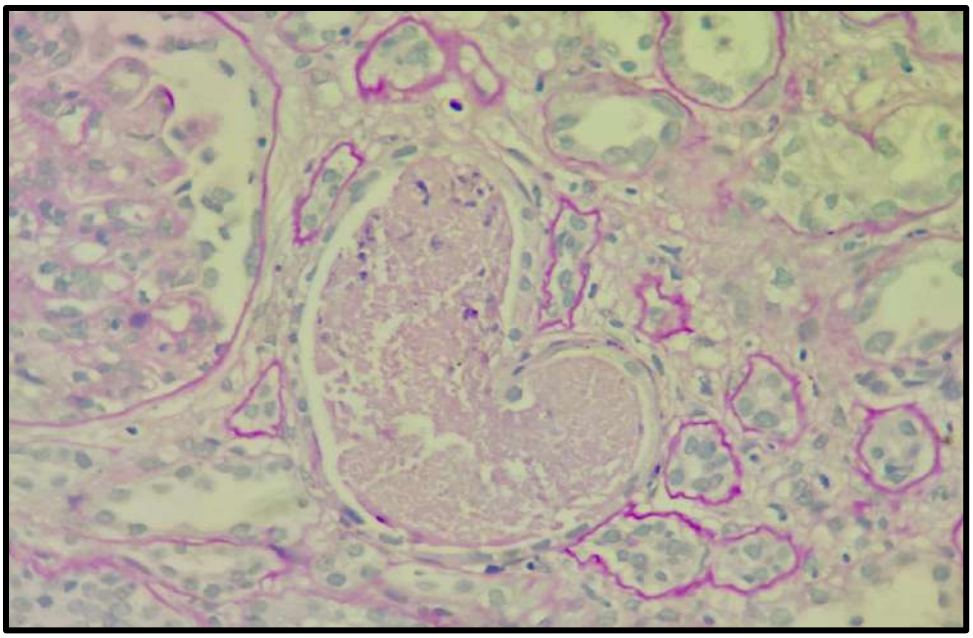
Picture 28- Photomicrograph showing a sclerosed glomerulus with periglomerular lymphomononuclear inflammatory infiltrate (MT, 400X)



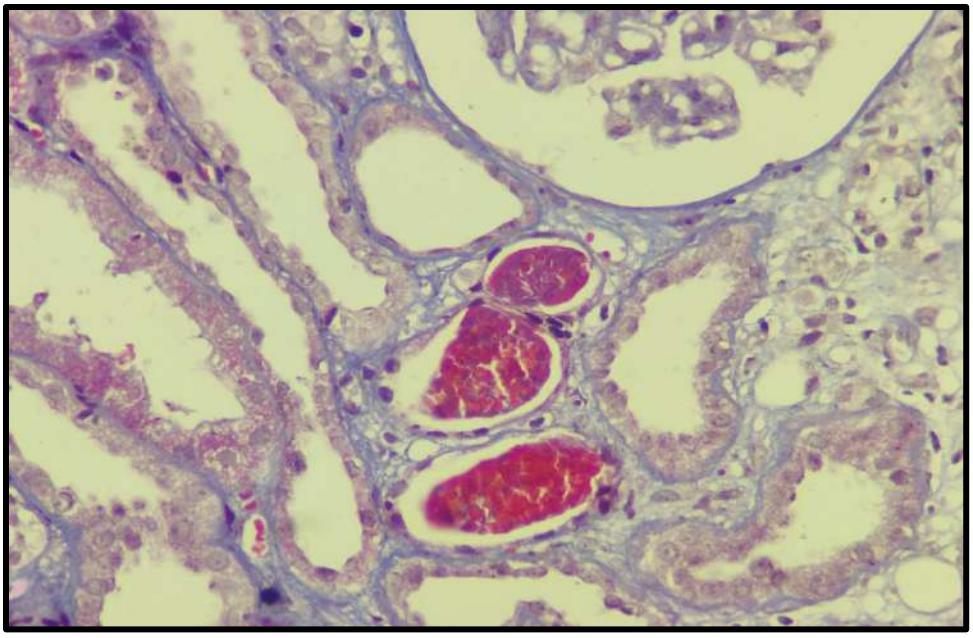
Picture 29- Photomicrograph showing 2 sclerosed glomeruli caught up in the interstitial scar and one viable glomerulus (MT, 100X)



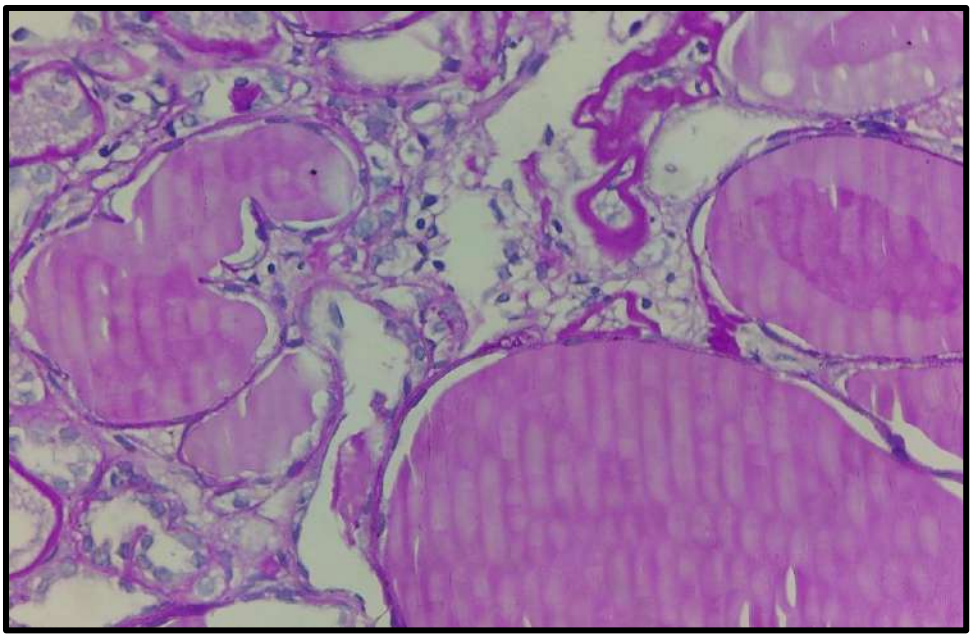
Picture 30- Photomicrograph showing periglomerular fibrosis (MT,400X)



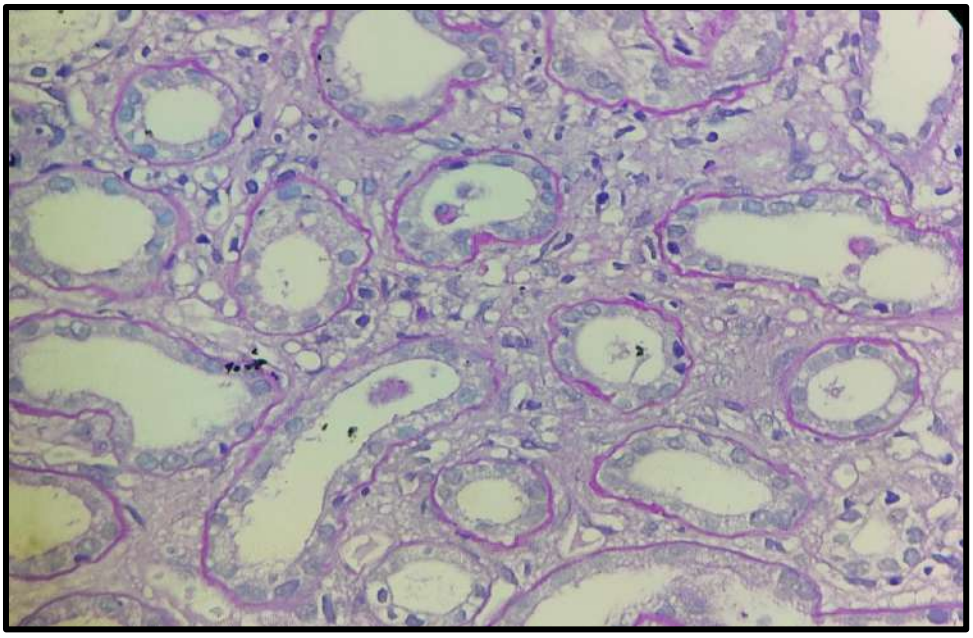
Picture 31- Photomicrograph showing granular cast in the tubules (PAS,400X)



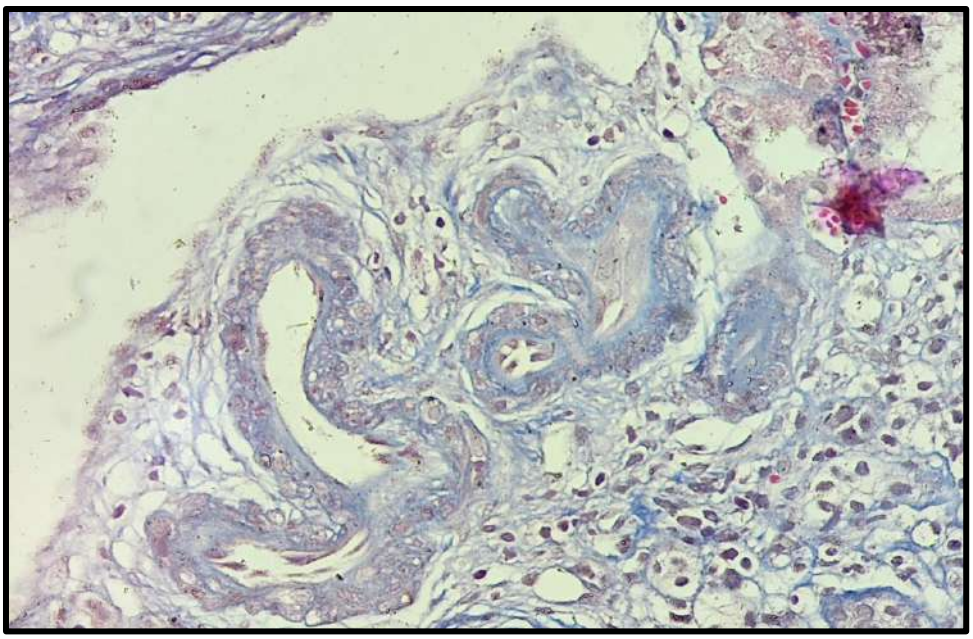
Picture 32- Photomicrograph showing RBC casts in the tubules (MT, 400X)



Picture 33- Photomicrograph showing hyaline casts in the tubules (PAS,400X)



Picture 34- Photomicrograph showing tubular atrophy (PAS,400X)



Picture 35- Photomicrograph showing intimal thickening of the vessel wall (MT,400X)

S No.	MRD	Inittial	Age	Age Group	Sex	Histopath No.	Malar Rash (acute)	Discoid Lupus (chronic)	Oral/Nasal ulcers	Alopecia	Synovitis	Serositis	Neurologic
1	924813	JK	40	21-40	F	20-3743	Absent	Absent	Absent	Absent	Present	Absent	Absent
2	922458	JK	32	21-40	F	20-3831	Absent	Absent	Absent	Absent	Absent	Absent	Altered sensorium
3	912411	R	35	21-40	F	20-5055	Present	Absent	Present	Present	Present	Absent	Absent
4	924520	BK	40	21-40	F	20-5575	Absent	Absent	Absent	Absent	Present	Present	Absent
5	948394	MK	42	41-60	F	20-5789	Absent	Absent	Absent	Absent	Absent	Absent	Absent
6	959825	PKT	28	21-40	F	20-6185	Absent	Absent	Present	Absent	Present	Absent	Absent
7	964524	P	31	21-40	F	20-6392	Absent	Absent	Absent	Present	Present	Present	Absent
8	1032309	SS	17	1-20	M	21-1785	Present	Absent	Absent	Present	Present	Absent	Absent
9	341098	NK	29	21-40	F	21-2125	Absent	Absent	Absent	Absent	Absent	Absent	Absent
10	1084848	JK	40	21-40	F	21-3451	Absent	Absent	Present	Present	Absent	Absent	Absent
11	997239	RS	68	61-80	M	21-3720	Absent	Absent	Present	Absent	Present	Present	Absent
12	1036738	AJ	7	1-20	M	21-4497	Present	Absent	Absent	Absent	Absent	Absent	Absent
13	1140039	S	45	41-60	F	21-6350	Present	Absent	Absent	Absent	Present	Absent	Absent
14	1151036	JK	41	41-60	F	21-7011	Absent	Absent	Absent	Present	Absent	Absent	Absent
15	1156258	PK	47	41-60	F	21-7463	Absent	Absent	Absent	Absent	Present	Absent	Absent
16	1157628	PV	29	21-40	F	21-7526	Present	Absent	Absent	Present	Absent	Absent	Absent
17	1157150	U	34	21-40	F	21-7889	Absent	Absent	Present	Absent	Present	Absent	Altered sensorium
18	1174490	Gk	35	21-40	F	21-8568	Absent	Absent	Absent	Absent	Present	Absent	Absent
19	1194592	OS	28	21-40	M	21-9957	Absent	Absent	Absent	Absent	Absent	Present	Absent
20	1196596	MK	29	21-40	F	21-10144	Present	Absent	Present	Present	Present	Absent	Absent
21	1080129	AK	73	61-80	F	21-11049	Present	Absent	Absent	Present	Absent	Absent	Absent
22	1169497	MS	38	21-40	M	21-11374	Present	Absent	Absent	Present	Absent	Absent	Absent
23	1217580	AS	29	21-40	F	21-11497	Absent	Absent	Absent	Absent	Absent	Present	Absent
24	1218174	D	28	21-40	F	21-11499	Absent	Absent	Present	Present	Present	Absent	Absent
25	1172671	CA	62	61-80	F	22-1377	Present	Absent	Absent	Absent	Present	Absent	Absent
26	1258048	NR	54	41-60	F	22-1779	Absent	Absent	Present	Present	Present	Absent	Absent
27	1242475	HK	52	41-60	F	22-1826	Present	Absent	Absent	Absent	Present	Absent	Absent

S No.	MRD	Oedema	Other	ANA	Anti dsDNA	Anti Sm	Anti nucleosome	Anti histone	Anti SS-A
1	924813	Present	Pain abdomen	Positive	Strong positive	Strong positive	Positive	Positive	Positive
2	922458	Absent	Hematuria	Positive	Negative	Negative	Negative	Negative	Positive
3	912411	Absent	Frothy urine, SOB	Positive	Negative	Negative	Negative	Negative	Negative
4	924520	Absent	Frothy urine	Positive	Strong positive	Positive	Positive	Positive	Positive
5	948394	Present	Menstrual irregularities	Positive	Negative	Negative	Negative	Negative	Positive
6	959825	Present	Absent	Positive	Positive	Positive	Positive	Positive	Negative
7	964524	Present	Weakness, fever	Positive	Positive	positive	Positive	Positive	Positive
8	1032309	Absent	Absent	Positive	Positive	positive	Positive	Negative	Negative
9	341098	Present	Hematuria	Positive	Positive	Positive	Negative	Negative	Negative
10	1084848	Absent	Absent	Positive	Positive	Positive	Positive	Negative	Negative
11	997239	Present	Absent	Positive	Negative	Negative	Negative	Positive	Negative
12	1036738	Absent	Periorbital swelling, Oliguria	Positive	Strong positive	Strong positive	Positive	Positive	Negative
13	1140039	Present	Hematuria, Frothy urine	Positive	Negative	Negative	Negative	Negative	Negative
14	1151036	Present	Absent	Positive	Negative	Negative	Negative	Negative	Negative
15	1156258	Present	SOB, Frothy urine	Positive	Positive	Strong positive	Positive	Positive	Positive
16	1157628	Absent	Frothy urine	Positive	Strong positive	Strong positive	Positive	Positive	Positive
17	1157150	Absent	Reduced appetite	Positive	Positive	Negative	Positive	Positive	Positive
18	1174490	Present	Pain abdomen	Positive	Strong positive	Negative	Negative	Negative	Negative
19	1194592	Absent	Oliguria, Fever	Positive	Strong positive	Negative	Positive	Positive	Negative
20	1196596	Absent	Pain abdomen	Positive	Strong positive	Negative	Positive	Positive	Negative
21	1080129	Absent	Absent	Positive	Negative	Negative	Negative	Negative	Negative
22	1169497	Absent	Absent	Positive	Positive	Weakly Positive	Negative	Negative	Negative
23	1217580	Present	Dyspnea	Positive	Negative	Negative	Positive	Negative	Negative
24	1218174	Absent	Absent	Positive	Positive	Positive	Negative	Negative	Negative
25	1172671	Absent	Absent	Positive	Positive	Negative	Negative	Negative	Negative
26	1258048	Absent	Absent	Positive	Positive	Negative	Negative	Negative	Positive
27	1242475	Present	Absent	Positive	Positive	Positive	Positive	Positive	Negative

S No.	MRD	Anti SS-B	Anti Scl 70	ANCA	Serum C3	Serum C4	Hb	TLC	Platelets	ESR	Proteinuria	Urine RBC	Urine pus cells	Urine casts
1	924813	Positive	Negative	Negative	Reduced	Reduced	7.5	3.8	154	56	2+	20-22/hpf	Nil	Nil
2	922458	Positive	Negative	Not done	Reduced	Reduced	8.6	4.8	323	27	3+	8-10/hpf	50-60/hpf	Nil
3	912411	Negative	Negative	Not done	Reduced	Normal	8.6	9.1	276	46	3+	1-2/hpf	Nil	Nil
4	924520	Negative	Negative	Not done	Reduced	Reduced	7.7	5.6	388	108	1+	40-45/hpf	Nil	Nil
5	948394	Positive	Negative	Not done	Normal	Reduced	10.4	9.8	342	98	3+	Nil	Nil	Nil
6	959825	Negative	Negative	Not done	Reduced	Reduced	6.9	4	140	65	2+	3-4/hpf	10-15/hpf	Nil
7	964524	Positive	Negative	Not done	Normal	Normal	8	2.4	327	120	2+	Nil	3-5/hpf	Nil
8	1032309	Negative	Negative	Not done	Reduced	Reduced	9.8	16.2	158	13	3+	15-20/hpf	Nil	Nil
9	341098	Negative	Negative	Not done	Reduced	Reduced	9.4	6	382	86	3+	10-12/hpf	5-7/hpf	Nil
10	1084848	Negative	Negative	Not done	Reduced	Reduced	12.3	10.3	167	46	Traces	5-7/hpf	Nil	Nil
11	997239	Negative	Negative	Negative	Reduced	Reduced	10.5	8.7	164	48	3+	90-100/hpf	2-3/hpf	Nil
12	1036738	Negative	Negative	Not done	Reduced	Reduced	10.8	19.8	201	45	2+	50-60/hpf	Nil	Nil
13	1140039	Negative	Negative	Positive (p)	Reduced	Normal	6.6	7.2	173	75	3+	30-40/hpf	Nil	Nil
14	1151036	Negative	Negative	Not done	Reduced	Normal	7.2	7	261	80	3+	60-70/hpf	Nil	Nil
15	1156258	Negative	Negative	Negative	Reduced	Normal	10.3	8	257	85	3+	2-4/hpf	Nil	Nil
16	1157628	Positive	Negative	Not done	Reduced	Reduced	9.4	4.5	161	52	3+	Nil	Nil	Nil
17	1157150	Positive	Negative	Not done	Reduced	Reduced	9.8	1	80	26	3+	2-3/hpf	5-7/hpf	Nil
18	1174490	Negative	Negative	Not done	Reduced	Reduced	9	3	116	78	3+	3-5/hpf	8-10/hpf	Nil
19	1194592	Negative	Negative	Not done	Reduced	Reduced	12.1	8.1	84	58	3+	20-25/hpf	Nil	Nil
20	1196596	Negative	Negative	Not done	Reduced	Reduced	10.4	4	116	42	3+	8-10/hpf	Nil	Nil
21	1080129	Negative	Negative	Negative	Normal	Normal	8.9	8.7	329	56	3+	20-25/hpf	Nil	Nil
22	1169497	Negative	Negative	Not done	Normal	Normal	10.8	7	227	49	3+	Nil	4-5/hpf	Nil
23	1217580	Positive	Negative	Negative	Reduced	Normal	8.3	11.5	348	45	3+	15-20/hpf	Nil	Nil
24	1218174	Negative	Negative	Not done	Normal	Normal	10.1	3	210	60	3+	5-6/hpf	22-25/hpf	Nil
25	1172671	Negative	Negative	Negative	Normal	Normal	8.5	16.8	326	92	3+	20-25/hpf	40-50/hpf	Nil
26	1258048	Positive	Negative	Positive (p)	Reduced	Normal	10.2	4.3	161	45	2+	6-8/hpf	Nil	Nil
27	1242475	Negative	Negative	Not done	Reduced	Reduced	8.8	5.8	182	47	3+	30-32/hpf	10-15/hpf	Nil

S No.	MRD	Spot Urinary protein mg/dl	urinary creatinine mg/dl	Urinary Protein: creatinine	Blood urea mg/dl	Serum creatinine mg/dl	Total proteins g/dl	Albumin g/dl	Focal / Diffuse	Mesangial hypercellularity	Endocapillary hypercellularity	Leucocytic infiltration
1	924813	141.6	146.1	1	53	0.89	8.1	2.83	Diffuse	Present	Present	Present
2	922458	999.6	77.9	12	102	1.89	4.5	2.02	Diffuse	Present	Absent	Present
3	912411	860.8	106.6	8	62	2.96	6.6	2.95	Diffuse	Present	Absent	Absent
4	924520	269.3	0.99	2.1	44	0.94	5.5	2.3	Focal	Present	Present	Present
5	948394	380.2	10.4	5.5	46	0.38	4.3	2.46	Diffuse	Present	Absent	Absent
6	959825	181.1	2.2	1.76	57	0.88	6.7	2.57	Diffuse	Present	Present	Present
7	964524	451.5	47.3	9.5	157	4.53	4.6	2.14	Diffuse	Present	Present	Present
8	1032309	1164.4	329.9	3.3	23	0.58	6.8	3.76	Focal	Present	Present	Present
9	341098	192.3	3.1	1.7	27	0.88	5.8	3.2	Focal	Present	Present	Present
10	1084848	76	105.7	0.71	37	0.75	6	3.42	Diffuse	Present	Absent	Present
11	997239	160.4	37.9	4.2	58	2.5	5.8	3	Diffuse	Present	Present	Present
12	1036738	111.4	71.7	1.6	41	0.29	4.8	2.25	Focal	Present	Absent	Present
13	1140039	349.4	40.5	8.62	96	4.98	6	2.9	Diffuse	Present	Present	Present
14	1151036	352.9	30.9	11.4	59	4.28	5.6	2.9	Diffuse	Present	Present	Present
15	1156258	366.9	28.1	13	206	1.64	5.1	1.32	Focal	Present	Present	Present
16	1157628	689.7	121.1	5.69	24	0.7	5.1	1.91	Focal	Present	Present	Present
17	1157150	23.2	8.2	2.8	45	0.36	5.7	2.43	Focal	Present	Absent	Absent
18	1174490	38.3	30.1	1.27	25	0.61	4.9	3.2	Diffuse	Present	Present	Present
19	1194592	285.9	101.6	2.81	217	2.53	3.5	1.61	Diffuse	Present	Absent	Present
20	1196596	185.5	4.2	1.8	23	0.34	7.2	3.39	Focal	Present	Present	Present
21	1080129	407.1	37.7	10.7	74	2.3	6	3.08	Diffuse	Present	Present	Present
22	1169497	13.6	49.6	0.27	18	2	6.2	3.1	Diffuse	Present	Absent	Absent
23	1217580	474.3	164.6	2.88	24	1.4	3.8	1.9	Diffuse	Present	Present	Present
24	1218174	868.1	201.7	4.3	26	0.85	5.4	3.1	Diffuse	Present	Absent	Absent
25	1172671	199.9	18.4	10.8	220	5.05	5.1	2.88	Diffuse	Present	Present	Present
26	1258048	111.5	16.6	6.7	60	3.91	5.8	2.6	Diffuse	Present	Absent	Present
27	1242475	98.1	55.2	1.77	41	0.81	5	2.7	Diffuse	Present	Present	Present

S No.	MRD	Crescents	Basement membrane thickening	Fibrinoid necrosis	Wire loop lesions	karryorhexis	hyaline deposits	Tubular atrophy	Interstitial Inflammation	Fibrosis IFTA %
1	924813	Present (C)	Absent	Present	Absent	Present	absent	Present	Chronic	20
2	922458	Present (FC,F)	Present	Present	Absent	Absent	absent	Present	Mixed	10
3	912411	Present (F)	Present	Absent	Absent	Absent	absent	Present	chronic	60
4	924520	Present (C)	Absent	Absent	Absent	Present	absent	Present	chronic	30
5	948394	Absent	Present	Absent	Absent	Absent	absent	Present	chronic	25
6	959825	Present (C, FC, F)	Present	Absent	Absent	Present	absent	Absent	chronic	<10%
7	964524	Present (C, FC)	Absent	Absent	Absent	Present	absent	Present	Mixed	Absent
8	1032309	Absent	Present	Absent	Present	Present	absent	Absent	Absent	Absent
9	341098	Cellular	Absent	Absent	Absent	present	absent	present	chronic	Absent
10	1084848	present (C, FC)	Present	Absent	Absent	Absent	absent	present	chronic	10
11	997239	Present (C, FC,F)	Present	Present	Present	Present	absent	present	Chronic	40-50
12	1036738	Absent	Absent	Absent	Absent	Absent	absent	Present	Mixed	Absent
13	1140039	Present (C)	Present	Absent	Present	Absent	absent	Present	Mixed	10
14	1151036	Present (C,FC)	Present	Absent	Present	Present	absent	present	Mixed	5-10
15	1156258	Present (C,FC)	Absent	Absent	Absent	Absent	absent	Present	Mixed	5-10
16	1157628	Absent	Present	Absent	Absent	Present	absent	Present	Absent	Absent
17	1157150	Absent	Absent	Absent	Absent	Absent	absent	Absent	Absent	Absent
18	1174490	Present (FC,C)	Present	Present	Absent	Present	absent	Absent	Absent	5-10
19	1194592	Absent	Present	Absent	Absent	Present	present	Absent	Absent	Absent
20	1196596	Absent	Present	Present	Absent	Present	absent	Absent	Absent	Absent
21	1080129	Absent	Absent	Absent	Absent	Absent	absent	Present	Chronic	15
22	1169497	Absent	Present	Absent	Absent	Absent	absent	Absent	Absent	Absent
23	1217580	Present (C,FC)	Absent	Present	Absent	Present	absent	Present	Mixed	Absent
24	1218174	Absent	Absent	Absent	Absent	Absent	absent	Absent	Absent	Absent
25	1172671	Absent	Absent	Absent	Present	Absent	absent	Present	chronic	55-60
26	1258048	Present (C, FC, F)	Absent	Absent	Absent	Present	absent	Present	Mixed	10%
27	1242475	Absent	Absent	Absent	Absent	Present	absent	Present	chronic	10

S No.	MRD	Vascular lesions	Modified Activity Index	Modified chronicity Index	DIF	ISN/RPS class	Pattern
1	924813	Myointimal hyperplasia	11/24	3/ 12	IgG, IgA, C3c, C1q	IV	Diffuse LN
2	922458	hyaline arterisclerosis	8/24	4/ 12	IgG, IgA	IV	Diffuse LN
3	912411	intimal sclerosis	2/24	10/ 12	Full house positivity	III+V	Membranous LN
4	924520	Unremarkable	8/24	05/ 12	IgG, C3c, IgM, C1q	III	Focal LN
5	948394	Mild medail hypertrophy	1/24	02/ 12	IgG, C1q, Kappa Lambda	V	Membranous LN
6	959825	Unremarkable	7/24	01/ 12	Full house positivity	IV	Diffuse LN
7	964524	Intimal fibrosis	13/24	0/12	Full house positivity	IV	Diffuse LN
8	1032309	Mild medial atrophy	2/24	0/12	Full house positivity	III+V	Membranous LN
9	341098	Mild myointimal hyperplasia	8/24	0/12	Full house positivity	IV	Diffuse LN
10	1084848	Unremarkable	4/24	3/ 12	Full house positivity	IV	Diffuse LN
11	997239	Medial thickening	9/24	6/ 12	IgG, IgM, C3c	IV	Crescentic LN
12	1036738	Unremarkable	2/24	1/ 12	Full house positivity	III	Mesangial proliferative LN
13	1140039	Unremarkable	4/24	1/ 12	IgG,C3c,	III+V	Membranous LN
14	1151036	medial expansion	8/24	3/ 12	IgG, C3c, C1q	IV	Diffuse LN
15	1156258	Mildly thickened walls	7/24	3/ 12	Full house positivity	III	Focal LN
16	1157628	Unremarkable	3/24	0/12	Full house positivity	III	Focal LN
17	1157150	mild medail atrophy	0/24	0/12	Full house positivity	II	Mesangial proliferative LN
18	1174490	Unremarkable	16/24	2/ 12	Full house positivity	IV	Diffuse LN
19	1194592	Unremarkable	5/24	0/12	Full house positivity	V	Membranous LN
20	1196596	Mild intimal thickening	5/24	0/12	Inadequate	III	Focal LN
21	1080129	Unremarkable	5/24	2/ 12	Full house positivity	IV	Diffuse LN
22	1169497	Unremarkable	0/24	0/12	Full house positivity	V	Membranous LN
23	1217580	Unremarkable	13/24	1/ 12	Full house positivity	IV	Crescentic LN
24	1218174	Unremarkable	0/24	0/12	Full house positivity	II	Mesangial proliferative LN
25	1172671	Intimomedial thickening	2/24	9/ 12	No deposits	VI	Advanced sclerosing LN
26	1258048	Hyaline arteriosclerosis	7/24	5/ 12	Full house positivity	IV	Crescentic LN
27	1242475	Myointimal expansion	4/24	04/ 12	Full house positivity	IV	Diffuse LN

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No. BFUHS/2K21p-TH/ 203 Date 5/1/2022

The Principal,
DMC, Ludhiana.

Subject: **Approval of Plan of Thesis.**

This is to inform you that the Faculty of Medical Sciences in its meeting held on 29.10.2021 has approved the plan of thesis of **Ramneek Kaur**, a student of your college for **MD Pathology** along with the names of Supervisor/Co-Supervisor as under:-

Topic Plan of Thesis
Morphological spectrum of lupus nephritis.

Supervisor
Dr. Sumit Grover
Assoc. Prof.,
Dept. of Pathology,
DMCH, Ludhiana.

Co-Supervisor

Dr. Pavneet Kaur Selhi
Prof.,
Dept. of Pathology,
DMCH, Ludhiana.

Dr. Simran Kaur
Asst. Prof.,
Dept. of Nephrology,
DMCH, Ludhiana.

The student has to submit the four copies of thesis (along with PDF format) , to the Controller of Examinations, BFUHS, Faridkot, at least six months before the commencement of theory examination, if he/she is otherwise eligible to take the examination. He /She must directed to attach a photocopy of this letter with the forwarding letter while submitting thesis to the University along with the letter of approval of University for change of supervisor/Co-Supervisor, change of topic,(if any).

Dr. S P Singh MS(Oph)
Controller of Examinations

Note :

101. If any discrepancy found regarding topic of thesis or Supervisor/Co-Supervisor, inform undersigned within one month after issue of this letter.

102. The student may submit the thesis as per the schedule already conveyed i.e for May/June examination 30th Nov of the preceding the year of examination and for Nov/Dec examinations 31st May of the year of examination.

MORPHOLOGICAL SPECTRUM OF LUPUS NEPHRITIS

THESIS PROPOSAL

FOR APPROVAL OF SUBJECT OF THESIS TO BE SUBMITTED IN

PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MD

IN

PATHOLOGY

OF THE

BABA FARID UNIVERSITY OF HEALTH SCIENCES

FARIDKOT

MARCH, 2021

DR. RAMNEEK KAUR

DEPARTMENT OF PATHOLOGY

DAYANAND MEDICAL COLLEGE AND HOSPITAL

LUDHIANA

ABSTRACT OF PLAN OF THESIS

Title	MORPHOLOGICAL SPECTRUM OF LUPUS NEPHRITIS
For the degree of	MD (PATHOLOGY)
Name of the candidate	Dr. Ramneek Kaur
Supervisor	Dr Sumit Grover, Associate Professor, Department of Pathology, DMCH, Ludhiana
Co-supervisor(s)	Dr Pavneet Kaur Selhi, Professor, Department of Pathology, DMCH, Ludhiana Dr Simran Kaur, Assistant Professor, Department of Nephrology, DMCH, Ludhiana
Institution	Dayanand Medical College and Hospital, Ludhiana
University	Baba Farid University of Health Sciences, Faridkot

Introduction: Renal disease manifests in up to 40-75% of SLE patients, most often occuring within five years of disease onset and hence is considered one of the strongest predictors of an unfavourable outcome. Renal biopsy at the time of initial presentation and subsequently during follow up may be needed to decide the therapeutic approach. Histopathological features play a significant role in patient management.

Aims & Objectives: 1. To study the histopathological features of kidney biopsies in patients of SLE. 2. To correlate the histopathological features with the clinical profile

Materials and Method: This study will be conducted in the Department of Pathology at Dayanand Medical College and Hospital, Ludhiana for a period of 2 years, of which 1 year (1st March 2020-28th Feb 2021) will be retrospective and 1 year (1st March 2021-28th Feb 2022) will be prospective. All the biopsies will be histologically analysed and classified into various classes based on light microscopy findings using routine, special stains and direct immunofluorescence. Correlation with indirect immunofluorescence findings will be done whenever possible. In the retrospective analysis, the slides will be retrieved from the archives along with their clinical history and immunofluorescence finding.

Clinical Significance: To obtain a good database for assessment and diagnosis of Lupus Nephritis based on the histomorphological findings.

Keywords: Lupus Nephritis, Kidney Biopsy, EULAR classification, ANA, Direct immunofluorescence.

(Signature)	(Signature)	(Signature)	(Signature)	(Signature)
Candidate	Co-supervisor	Co-supervisor	Supervisor	Principal

CERTIFICATE OF FACILITIES AVAILABLE

This is to certify that the facilities for work on the subject of thesis titled **"MORPHOLOGICAL SPECTRUM OF LUPUS NEPHRITIS"** exist in the Department of Pathology, DMC&H, Ludhiana and will be provided to the candidate. We will see that the data being included in the thesis is genuine and is collected by the candidate herself under our supervision and guidance.

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Date : 16/3/2021

APPROVAL PROFORMA
BY RESEARCH & ETHICAL COMMITTEE
DAYANAND MEDICAL COLLEGE & HOSPITAL, LUDHIANA

Name of candidate	Dr. Ramneek Kaur
Department	Pathology
Topic of Thesis	MORPHOLOGICAL SPECTRUM OF LUPUS NEPHRITIS
Likely date of appearing in PG examination	May 2023
Date of enrollment	27th July, 2020
Name of Head of Department	Dr. Harpreet Kaur
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Signature of Members of Research Committee with Stamp	**Signature of Members of Ethics Committee with Stamp**
Dr. Harpreet Kaur (Professor & Head, Pathology)	Mr. R K Sharma (Legal Expert)
Dr. Jaspal Singh (Professor & Head, Surgery)	Dr. Ramit Mahajan (Associate Professor, Gastroenterology)
Dr. Dinesh Gupta (Professor & Head, Medicine)	Dr. Anu Sharma (Professor, Anatomy)
Dr. Sandeep Kaushal (Dean Academics & Professor & Head, Pharmacology)	Dr. Sandeep Kaushal (Dean Academics & Professor & Head, Pharmacology)
Dr. Gurpreet S Wander (Vice Principal)	Dr. Gurpreet S Wander (Vice Principal & Professor & Head, Cardiology)
	Dr. Gagandeep Singh (Secretary, Ethics Committee Professor & Head, Neurology)

Approved: Yes / No

Dr. Sandeep Puri
Chairperson
Research Committee

Approved: Yes / ~~No~~

Prof. Arvind Malhotra
Chairperson
Ethics Committee

BABA FARID UNIVERSITY OF HEALTH SCIENCES, FARIDKOT

PROFORMA FOR REGISTRATION OF SUBJECT FOR DISSERTATION

1	NAME OF THE CANDIDATE AND ADDRESS	RAMNEEK KAUR D/O HARCHARAN SINGH #126 BHARAT NAGAR BATHINDA, PUNJAB 151001
2	NAME OF THE INSTITUTE	DAYANAND MEDICAL COLLEGE AND HOSPITAL, LUDHIANA
3	COURSE OF STUDY AND SUBJECT	MD PATHOLOGY
4	DATE OF ADMISSION OF COURSE	27 JULY 2020
5	TITLE OF THE TOPIC	MORPHOLOGICAL SPECTRUM OF LUPUS NEPHRITIS

6. BRIEF RESUME OF THE INTENDED WORK

6.1 NEED FOR THE STUDY

Systemic lupus erythematosus (SLE) is a multisystem inflammatory disorder which can affect almost any organ in the body. The involvement of these organs can manifest in more than one way. More commonly it involves systems like the central and peripheral nervous systems, lungs, heart, skin, serous membranes, hematological system, and the kidneys.[1] With diverse manifestations of SLE across different patients, the diagnosis may be challenging. SLE has a significant impact across the world among different ages, and racial groups more commonly affecting young and middle aged women.[2]

When the kidneys are affected by SLE the condition is known as Lupus Nephritis, the extent of which may vary between individuals.[3] Lupus nephritis (LN) being one of the most common and devastating manifestations of SLE, affects over half of patients with this systemic disease.[4] Most of the patients with SLE develop nephritis early in the course of their disease where the vast majority of patients are younger than 55 years, while children are more likely to develop severe nephritis than the elderly patients.[5]

Antinuclear antibodies (ANA) are the hallmark of the disease and is usually the initial test performed. Immunofluorescence assay is considered the gold standard test for ANA, and although other methods of detection such as ELISA and multiplex assays are widely available, they lack sensitivity. Anti-Ds-DNA antibodies have more than 95% specificity for SLE but are seen in only about 60% to 70% of SLE patients. Anti-Ro (SSA) and Anti-La (SSB) antibodies target ribonucleoprotein particles. Anti-Ro and Anti-La antibodies are seen in up to 90% of cases of Sjogren syndrome but can be seen in SLE as well. Anti-Smith antibodies are seen in less than 30% of SLE patients but have 99% specificity for SLE.[6]

Around 40-75% of SLE patients present with renal diseases which most often occurs within five years of disease onset.[7] Hence, renal

biopsy at the time of initial presentation and subsequently while follow up may be needed to decide the therapeutic approach.[4]

The new classification criteria of SLE (2019 criteria), adapted by both the ACR and European League Against Rheumatism (EULAR) aim to bring the consensus on the histopathological front for the different Lupus Nephritic biopsy terminologies while standardizing the interpretation and reporting of the kidney biopsies between different centres.[8]

The new EULAR classification also aims at improving the specificity and sensitivity.[9] According to the European League Against Rheumatism and European Renal Association–European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations, in patients with GFR below 30 ml/min, the decision for biopsy becomes doubtful unless the kidney size is normal (> 9 cm length in adults) and/or there is evidence of renal disease activity.[10]

Being a department of a tertiary care hospital, we receive a lot of kidney biopsies of the patients with clinical suspicion of LN. Through this study we aim to analyse kidney biopsy received with the clinical suspicion of Lupus Nephritis and classify these into different classes as per the new ACR and EULAR guidelines. We also aim to correlate the histopathological features with clinical and serological features of the patients.

6.2 REVIEW OF LITERATURE

Renal disease manifests in up to 40-75% of SLE patients, most often occurring within five years of disease onset and hence is considered one of the strongest predictors of an unfavourable outcome.[7]

The renal involvement presents mainly in the form of glomerulonephritis and tubulointerstitial nephritis. Nephritis was first described as a component of systemic lupus erythematosus by William Osler.[11] In SLE, kidney biopsy is indicated because active GN may be part of the initial acute presentation, or may present at the time of relapse during follow-up. Relapses occur relatively frequently and early detection is associated with better outcomes.[7]

The World Health Organization categorizes Lupus Nephritis into 6 classes, all with distinct pathological features and significant differences in clinical outcomes. This has a significant role in patient management in adapting a different treatment approach for each class.

- **Class I: Minimal mesangial lupus nephritis**- immune complex deposition in the mesangium which can be identified by immunofluorescence and by electron microscopy. No structural changes by light microscopy.
- **Class II: Mesangial proliferative lupus nephritis**-Mesangial cell proliferation without involvement of glomerular capillaries. Normal by light microscopy but has mesangial-positive IF changes and positive EM changes within the mesangium
- **Class III: Focal lupus nephritis**-involvement of fewer than 50% of glomeruli. Focal and segmental proliferative changes by light microscopy, positive IF and EM changes
- **Class IV: Diffuse segmental or diffuse global lupus nephritis**-involvement of >50% of glomeruli. “Wire loop” structures identified on Light microscopy are due to Subendothelial deposition of immune complexes. Diffuse proliferative glomerulonephritis with positive IF and EM
- **Class V: Membranous lupus nephritis**-diffuse thickening of the capillary walls due to deposition of immune complexes.

Features of diffuse membranous glomerulonephritis- subepithelial and mesangial immune deposits ("spike and dome" pattern with silver stain); nephrotic syndrome or severe proteinuria.

- **Class VI: Advanced sclerosing lupus nephritis**- sclerosis of >90% of the glomeruli and represents end stage renal disease.[6,12]

ISN/RPS CLASSIFICATION OF LUPUS NEPHRITIS (2004)

Class I: Minimal Mesangial Lupus Nephritis:

- Glomeruli normal by light microscopy (LM), mesangial deposits by immunofluorescence (IF) / electron microscopy (EM)

Class II: Mesangial Proliferative Lupus Nephritis:

- Mesangial hypercellularity and/or mesangial expansion by LM, immune deposits by IF / EM

Class III: Focal Lupus Nephritis:

- Active or inactive focal, segmental or global endo or extracapillary glomerulonephritis involving < 50% of glomeruli, immune deposits are subendothelial
- Subclassified as A (active lesions), A/C (active and chronic lesions), C (chronic lesions)

Class IV: Diffuse Lupus Nephritis:

- > 50% glomeruli with active / inactive diffuse, segmental, or global endo and/or extracapillary glomerulonephritis
- Diffuse subendothelial deposits can cause wire-loop appearance
- A (active lesions), A/C (active and chronic lesions), C (chronic lesions)
- Diffuse segmental if > 50% glomeruli show segmental lesions, diffuse global if > 50% show global lesions

Class V: Membranous Lupus Nephritis:

- Global or segmental involvement, subepithelial immune deposits
- May occur in combination with class III or IV

Class VI: Advanced Sclerosing Lupus Nephritis:

- > 90% globally sclerosed glomeruli

In 2016, a meeting of 18 members of an international nephropathology working group in Leiden, netherlands was held where they proposed recommendations and adjustments in order to improve the clarity of definitions and classification of glomerular lesions. They provided new definitions for mesangial hypercellularity, cellular, fibrocellular and fibrous crescents. They also eliminated the class IV-S and IV-G subdivisions of class IV of lupus nephritis. They also proposed the inclusion of fibrinoid necrosis as a specific descriptor in the activity index.[13]

The five main pathological types of renal microvascular lesions in LN include: (1) vascular immune complex deposits (ICD), (2) arteriosclerosis (AS), (3) thrombotic microangiopathy (TMA), (4) non-inflammatory necrotizing vasculopathy (NNV), and (5) true renal vasculitis (TRV). Some of the key mechanisms in the development of vascular lesions include the activation and dysfunction of endothelial cells along with the contribution of immune system dysfunction, especially the immune complex-induced vascular inflammation and antiphospholipid antibody-associated thrombotic events. These mechanisms however need to be further investigated.[14]

Raskovic et al in their study analysed kidney biopsies of 58 patients with biopsy proven lupus nephritis and found that the histopathological findings of the renal biopsies belonged to class I lesion in 3.45% of patients, class IIA in 24.14%, class IIB in 31.03%, class III in 12.07%, class IV in 24.14%, class V in 3.35%, and class VI in 1.72% of cases according to the WHO classification. They also observed that the values of serum creatinine were elevated in 25.86% of patients, while creatinine clearance rate was lower (below 80 ml/min) in 75% of cases.[15]

Rabbani et al in their study on kidney biopsies of 48 Lupus patients concluded that the main histological types as per WHO classification were class IV (n=27), class III (n=7) and class V (n=6). Immunofluorescence showed a predominantly granular pattern of IgG,

IgA and C3. Renal manifestations included renal failure (50%), microscopic hematuria (67%), active urine sediment (22%), and proteinuria (74%). Proteinuria was nephrotic range in 45% cases.[16]

In the study by Satirapoj et al, Class IV (61.5%) was the most common class on evaluation of renal histopathology. Crescentic change and hyaline thrombi were found in 2.9% and 14.7% of biopsies, respectively. The most common manifestations of renal involvement in SLE patients were observed to be edema (75.0%). Urine sediment corresponding to a nephritic picture (defined as > 5 RBCs or any RBC casts per high power field in urine) and hypertension (blood pressure > 140/90 mmHg) were reported in 60.0% and 42.5% of the cases respectively. The mean value of proteinuria was 2,152.3 ± 1,620.2 mg/day.[17]

The renal biopsy specimens of the 61 patients included in a Greek study by Ourania et al revealed diffuse proliferative glomerulonephritis (class IV) as the most encountered type of renal disease in 23 patients, followed by the membranous lupus nephritis (class V) in 18 patients, the focal proliferative nephritis (class III) in 15 patients, and mesangial proliferative lupus nephritis (class II) in 5 patients. There were no differences regarding the gender or immunological profile and the histological class of nephritis identified on the biopsy samples.[18]

Aringer et al.[9] developed a new classification criteria that was rigorously developed in 4 phases and jointly supported by European League Against Rheumatism (EULAR) and American College of Rheumatology (ACR). The criteria for SLE included obligatory positive ANA at least once and a score from weighted addition of 7 clinical and 3 immunological (antiphospholipid antibodies, complement proteins, SLE-specific antibodies) domains between 2 to 10. Patients with greater than or equal to 10 points were classified. They found out that the new criteria had a sensitivity of 96.1% and specificity of 93.4% in diagnosing the disease, better than previous ACR 1997 criteria and Systemic Lupus International Collaborating Clinics 2012 criteria.

Rijnink et. al. in the year 2017, tried to correlate the prognosis of lupus nephritis patients with the histopathological features on kidney biopsy

on the basis of EULAR criteria 2012. Their results showed that although ISN/RPS classes were poorly associated with the clinically relevant outcomes, prognostication in LN would benefit from the specific assessment of clinical variables and lesions currently obscured in the classification. The authors particularly suggested that an independent scoring should be done based on atleast fibrinoid necrosis, fibrous crescents and IF/TA to assess the risk of progressive renal dysfunction in addition to clinical findings.[19]

In the study published in 2019 by Suda et al[20] on 100 Japanese patients, the researchers aimed to study the 2019 ACR/EULAR criteria in a real world setting. They concluded that the 2019 criteria for the classification of SLE achieved modestly high sensitivity in real-world practice. They possibly misclassified the real SLE cases as non-SLE, especially if patients had low titre (< 1:80) of ANA.

Pons-Estel et al [21] studied the 2019 EULAR/ACR criteria on Latin American patients. The focus was on earlier patients' classification in comparison to 1982/1997 ACR or the 2012 SLICC criteria. Considering the 1982/1997 criteria as gold standard, the sensitivity was found to be 91.3% for 2019 EULAR/ACR criteria. This criteria allowed an earlier SLE patient classification in 7.4% (mean 0.67years) and 0.6% (mean 1.47 years) than the 1982/1997 ACR and the 2012 SLICC criteria, respectively.

6.3 AIMS AND OBJECTIVES OF THE STUDY

1. To study in detail the histopathological features of kidney biopsies in patients of SLE.
2. To correlate the histopathological features with the clinical profile.

7. MATERIALS AND METHODS

7.1 Source of Data

This study will be conducted for a period of 5 years, three and a half years retrospective(1st March 2017-28th Feb 2021) and one and a half years prospective(1st March 2021- 28th Feb 2022), in the department of Pathology, Dayanand Medical College and Hospital Ludhiana. All the kidney biopsies of clinically diagnosed SLE patients included in the study will be analysed with the reference to the light microscopic findings and immunofluorescence. Correlation with ELISA, immunoblot, indirect immunofluorescence findings will be done wherever possible.

7.2 METHOD OF COLLECTION OF DATA

INCLUSION CRITERIA

Kidney biopsies from clinically diagnosed SLE patients

EXCLUSION CRITERIA-

All the kidney biopsies of Non SLE patients

Methodology: All kidney biopsies satisfying the inclusion criteria received in the Department of Pathology over a period of 5 years will be analysed with reference to light microscopic and direct immunofluorescence findings and will be correlated with the clinical profile i.e. clinical presentation, diagnosis and laboratory parameters. All the biopsies will be histologically analysed and classified into various classes based on light microscopy findings using routine (H&E) and special stains. Correlation with indirect immunofluorescence findings will be done whenever possible.

In the retrospective analysis, the slides will be retrieved from the archives along with their clinical immunofluorescence findings.

Sections for light microscopy will be stained with Hematoxylin-Eosin, Periodic Acid Schiff, Masson's Trichrome, Methenamine silver and Congo Red if needed. The slides will be subjected to the histopathological examination under both low power (100X) and high power (400X). Step sections will be done whenever required. Interpretation of histopathological findings will be done

DURATION OF STUDY

Five years

Retrospective study - Four years. (1st March 2017- 28th Feb 2021)

Prospective study - One year (1st Feb 2021-28th Feb 2022)

OUTCOME

Morphological spectrum of Lupus Nephritis will be studied and its clinical correlation will be done.

Statistical Analysis

Data will be described in terms of range: mean ± standard deviation(±SD), median, frequencies (number of cases) and relative frequencies (percentages) as appropriate. All statistical calculations will be done using SPSS (Statistical Package for the Social Science) SPSS 21 version statistical program for Microsoft Windows.

7.3 DOES THE STUDY REQUIRE ANY INVESTIGATIONS OR INTERVENTIONS TO BE CONDUCTED ON PATIENTS OR OTHER HUMANS OR ANIMALS? IF SO, PLEASE DESCRIBE BRIEFLY.

No.

7.4 HAS ETHICAL CLEARANCE BEEN OBTAINED FROM YOUR INSTITUTION IN CASE OF 7.3?

Attached herewith

LIST OF REFERENCES

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PROFORMA

Serial No: Date:

Histopath No: Phone no:

Name: MRD No:

Age: Sex:

CRITERIA FOR CLASSIFICATION

CLINICAL MANIFESTATIONS

Skin:

Acute, subacute cutaneous LE (photosensitive, Malar, maculopapular, bullous)

Chronic cutaneous LE (discoid lupus, panniculitis, lichen plants-like, hypertrophic verrucous, chilblains)

Oral or Nasal Ulcers:

Nonscarring Alopecia:

Synovitis involving ≥ 2 joints:

Serositis (pleurisy, pericarditis):

Renal:

Prot/Cr ratio ≥ 0.5

RBC Casts

Neurologic:

Seizures, psychosis, Mononeuritis, myelitis, peripheral or cranial neuropathies, acute confusional state

Hemolytic anemia:

Leucopenia (<4000/mcl) or

Lymphopenia (<1000/mcl):

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Thrombocytopenia (<100000/mcl):

IMMUNOLOGIC MANIFESTATIONS

ANA>reference negative value

Anti-dsDNA> reference, if by ELISA 2x reference

Anti-Sm

Antiphospholipid (any of lupus anticoagulant, false positive RPR, anti-cardiolipin, anti-B glycoprotein I)

Low serum complement (C3, C4 or CH50)

Positive direct Coombs test in absence of haemolytic anemia

INVESTIGATIONS

Hemogram

Hb TLC DLC

Platelet count ESR

Urine Examination

Urine R/E- Physical:-

Chemical:-

Proteinuria-

Microscopy:-

RBC- Pus Cells-

Casts-

24 hours urinary protein(whenever possible)

Urinary Creatinine

Albumin: Creatinine ratio

Protein: Creatinine ratio

USG/CECT/KUB (if required)

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Biochemical

Blood Urea S. Creatinine

Total Proteins Albumin

LDH Haptoglobin

Na/K/Cl

Serology

Anti-ds DNA

ANA

LIGHT MICROSCOPY FINDINGS

Kidney Biopsy - Adequate/Inadequate

Glomeruli: Normal/Involved

Involvement: Focal/Diffuse Proliferative/Sclerosing

Mesangial hypercellularity/Endocapillary hypercellularity/ Leucocytic infiltration:

Crescents: Absent Present-Cellular/Fibrosing/Fibrosed

Basement Membrane: Normal/Thickened

Fibrinoid Necrosis: Present/Absent

Wire loop lesions: Present/Absent

Karyorrhexis

Tubules & Interstitium:

Tubular Atrophy

Inflammation

Fibrosis

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Vascular lesions:

Special Stains

Masson's trichrome

Congo Red

Periodic Acid Schiff

Methenamine silver

MODIFIED NIH ACTIVITY INDEX(0-24)	**SCORE**	**SCORE**
Endocapillary Hypercellularity	0-3	
Neutrophils/Karyorrhexis	0-3	
Fibrinoid Necrosis	(0-3)x2	
Hyaline deposits	0-3	
Cellular/fibrocellular Crescents	(0-3)x2	
Interstitial Inflammation	0-3	
Total	0-24	

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MODIFIED NIH CHRONICITY INDEX(0-12)	SCORE	SCORE
Total Glomerulosclerosis sclerosis	0-3	
Fibrous Crescents	0-3	
Interstitial Fibrosis	0-3	
Tubular Atrophy	0-3	
Total	0-12	

[*<25% glomeruli involved (1+), 25-50% glomeruli involved (2+), >50% glomeruli involved (3+)]

Direct Immunofluorescence findings (if any)

Indirect Immunofluorescence findings

Final Pathological Diagnosis

Signature of candidate **Signature of Supervisor**